THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 127 SEPTEMBER 1, 1939 No. 2

THE ELECTRO-SALTATORY TRANSMISSION OF THE NERVE IMPULSE AND THE EFFECT OF NARCOSIS UPON THE NERVE FIBER

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Received for publication March 17, 1939

Nervous transmission is generally believed to be effected through excitation of each section of the nerve by the activity of adjacent parts. Most investigators assume that the restimulating agent which excites the resting region may be electrical in nature. This view of electrical transmission has been considerably strengthened by the recent work of Hodgkin (1938).

In the present paper I have attempted, by means of micro-technique, to obtain conclusive experimental evidence for the theory of electrical transmission, and to establish a more complete conception of nervous transmission. The problem of "decrement" in the narcotized region of nerve is also discussed with special reference to the new experimental data.

METHODS. In all experiments, isolated single nerve-fibers (sciatic-gastrocnemius preparation) of the Japanese toad were used. The procedures of isolation and experimentation were essentially similar to those described in previous papers (Tasaki, 1939). For multipolar stimulation, the circuit reported previously was employed, with a slight modification in the position of the current reversers.

For determining the least interval between two stimuli necessary to give a summated muscular contraction, break induction shocks supplied by two inductoria were used; the secondaries were connected in series and the strength of the shock was controlled by precision resistances in the primary circuits. In a single-fiber preparation, a summated contraction is distinctly larger than an ordinary single twitch and can easily be distinguished by visual observation. The least interval for muscular sum-

mation can therefore be much more easily determined in a single-fiber preparation than in the ordinary nerve-muscle preparation.

RESULTS. *1. Transmission over an inexcitable node of Ranvier.* In a previous paper (Tasaki, 1939b), it was shown that, when an isolated single nerve-fiber is excited with tripolar electrodes (fig. 1, inset), the relation between the voltages (V and V') necessary to excite constitutes a "triangle" whose three sides represent excitation at three different nodes of Ranvier. The nerve-fiber is excited by an outwardly directed current through the plasma membrane at the node. Excitation at the middle node (N_0) between the two sets of "ridge-insulators" is represented by the straight line A which can be expressed by the equation $V + V' = constant$.

Experiments on the effect of narcosis immediately indicated that a nervous impulse is able to "jump over" a small number of electrically inexcitable nodes. In a freshly isolated nerve-fiber, narcosis of a single node does not block nervous transmission through the narcotized region even when the narcotic is fairly concentrated. Increase in the concentration of the narcotic apparently did not deepen the narcosis. But when the same narcotic was allowed to act upon a stretch of nerve-fiber including a number of nodes, transmission through the narcotized region always ceased within a few seconds.

Although narcosis of a single node fails to block transmission, it brings about a definite and remarkable change in the excitability of the nerve-fiber, and this change can easily be disclosed by the method of tripolar stimulation. If the narcotic is sufficiently concentrated, *the side of the "triangle" corresponding to excitation at the narcotized node disappears*, while the two remaining sides are almost unaffected.

Figure 1 shows an example. Before narcosis, the usual triangle was obtained; but when a 2.5-per-cent urethane-Ringer solution was applied to the stretch of fiber between the two sets of ridge-insulators, side A of the triangle was extinguished and extensions of sides B and C appeared in the first quadrant.

The outwardly directed current through the plasma membrane at the node N_0 increases with increasing values of $(V+V')$, but there is no sign of excitation at this node. The threshold is ultimately reached by the current flowing outward through the node N_1 or N_2. At these nodes, the excitatory effect of the applied potential-differences is determined by the values $(V' \cot\beta - V)$ and $(V \cot\gamma - V')$ respectively, where β and γ represent the angles which sides B and C make with the horizontal and vertical axes. As shown in a former paper (Tasaki, 1939b), the plasma membrane at the node may be conceived as a semipermeable membrane of relatively high electric resistance, and, since the myelin sheath is a practically complete insulator, the potential-difference V' applied between the node N_2 and N_0 is capable of exciting the node N_1 if it is made sufficiently

great. The value of $\cot\beta$, which represents the effectiveness of the potential-difference V' in exciting the node N_1, depends mainly upon the polarization resistance through the node N_0; we may therefore take it as an *index of polarizability of the plasma membrane* at this node. It should be noted that the polarizability thus measured increases with increasing duration of the stimulating current employed for measurement.

In figure 2, the effect of weak narcosis is also shown. As the threshold at the node N_0 is raised by narcosis, side A of the triangle is shifted. The value $(V + V')$ increases with increasing concentration of the narcotic.

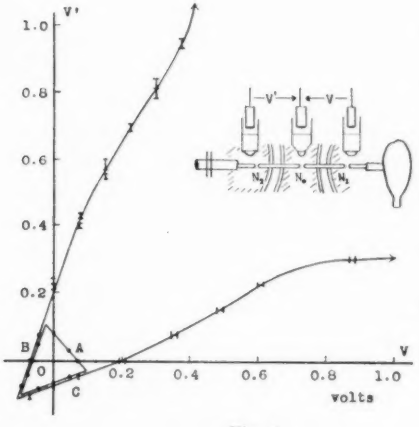

Fig. 1

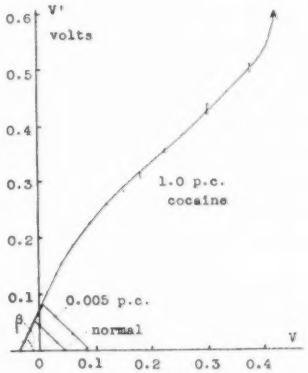

Fig. 2

Fig. 1. Tripolar stimulation of a nerve-fiber during narcosis of a single node of Ranvier. A 2.5 per cent urethane-Ringer solution was used. Duration of stimulating current, 0.5 msec. Temperature, 11.5°C. The small triangle shows the results obtained before narcosis. Side *A* of the triangle corresponds to excitation at the node N_0, side *B* to excitation at the node N_1, and side *C* at N_2.

Fig. 2. A similar experiment done with a cocaine solution. Pulse duration of stimulating currents, 1.0 msec. Temperature, 11.0°C. Conduction through the narcotized region was preserved.

When $(V + V')$ is two or three times the normal (at about 1.7 per cent for urethane and about 0.01 per cent for cocaine), determination of this side of the triangle becomes very uncertain and soon this line disappears completely. It is interesting that, when the excitability of the node N_0 is reduced or completely abolished by narcosis, the polarizability of this node remains unchanged.

As can be seen in the figures, the extension of the straight line corresponding to excitation at the node N_1 or N_2 loses its linearity when the current flowing through the inexcitable node N_0 is increased. For voltages

greater than about one volt, the threshold becomes unstable and rapidly rises, and it is difficult to determine the exact course of the line.

Only rarely does narcotization of a single node bring about reversible suspension of conduction. This seems to occur more often in preparations which have been maltreated. In that case, not only side A of the triangle but also side C disappears. With extremely concentrated narcotics (over 5 per cent in cocaine and over 10 per cent in urethane), conduction through the narcotized region is always suspended. This suspen-

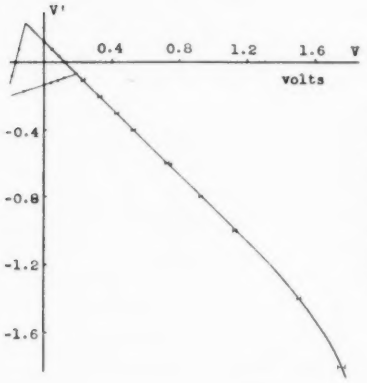

Fig. 3

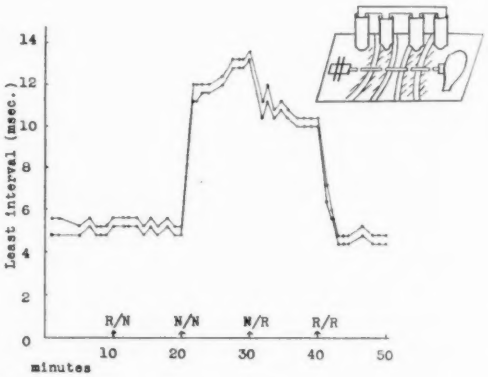

Fig. 4

Fig. 3. A 1.0 per cent cocaine-Ringer solution was introduced into the proximal pool. Conduction through the narcotized region was blocked. Pulse duration, 0.2 msec. Temperature, 9.5°C.

Fig. 4. Changes in the least interval for muscular summation produced by narcosis of nodes of Ranvier. Stimuli were applied about 3 cm. proximal to the isolated region. At the 10th minute from the onset of the experiment, a narcotizing solution (2.5 per cent urethane) was introduced into the distal small pool. At the 20th minute, the narcotic was also introduced into the proximal small pool. At the 30th minute, the narcotic in the distal pool was removed and was replaced with Ringer. At the 40th minute, the narcotic in the proximal pool was also removed. Distances between two sets of points in the figure indicate the accuracy of the measurements. Temperature, 10°C.

sion is usually irreversible and disintegration of the protoplasm seems to set in.

In the experiment of figure 3, Ringer's fluid in the proximal pool in which the node N_1 and the intact portion of the nerve were immersed was replaced with a concentrated narcotizing solution. Transmission through the narcotized region was of course suspended. In this case, side C of the triangle corresponding to excitation at the node N_2 was extinguished while the other two sides, A and B, remained unchanged. The extension of

side A of the triangle was perfectly linear for a wide range of voltage. It is clear from this and other experiments that each side of the triangle represents the excitability of a particular node and is not affected by narcosis of the neighboring nodes.

We may now conclude that a nervous impulse can pass beyond an electrically inexcitable node. This naturally leads us to assume a "jump" of the impulse over a short inactive region of nerve fiber. The objection may be raised, however, that the axis-cylinder (or the fibrils) may transmit the impulse even when the plasma membrane at the node is entirely inexcitable to artificial stimuli. The following experiments on the least interval for muscular summation answer this objection.

2. The least interval for muscular summation and the number of nodes of Ranvier exposed to the narcotic. When the least interval for muscular summation was measured, with electrodes on the central nerve-trunk, before and after application of narcotic to a stretch of nerve-fiber including only a single node, it was frequently observed that such a local narcosis brought about no detectable change in the least interval. If some change was produced, the change was not increased by increasing the concentration of the narcotic. It was surprising to find that the least interval remained practically unaltered when the concentration of cocaine was increased step by step from 0.01 per cent up to 1.0 per cent.

Although the least interval for muscular summation is almost independent of the concentration of narcotic, it depends most significantly upon the number of narcotized nodes. Even when narcosis of a single node produces no measurable change, narcosis of a stretch including two neighboring nodes causes a distinct prolongation. This is clearly shown by the experimental results in figures 4 and 5.

Ringer's fluid on a glass plate was divided into four pools with three sets of ridge-insulators (fig. 4 top) and all the pools were connected electrically with one another by means of a "salt-bridge" consisting of glass tubes filled with gelatin-Ringer gel. The narcotic was applied to the nerve-fiber in one of the middle pools or in both of them. In all cases, the least interval was distinctly longer when two nodes were narcotized than when a single node was exposed to the narcotic. In some favorable cases it happened that an impulse set up by a single induction shock could travel beyond one inexcitable node but not beyond two. The recovery of the least interval after the narcotizing solution was replaced by Ringer solution was in general almost complete except when a very concentrated cocaine solution was used.

It should be noted that in these experiments there is always a non-narcotized (desiccated) region in the nerve-fiber between the two small pools. As diffusion is a very slow process, we may assume that this desiccated region of the nerve-fiber (about 1.2 mm. in length) is not affected by

the narcotic. If transmission of the nerve impulse depended upon some continuous process along the axis-cylinder, an impulse which passed beyond the first narcotized stretch would regain its full intensity on emerging into this non-narcotized region, and such an impulse could not be blocked by the second narcotized region. Since blocking actually occurs, we may conclude that the impulse is not transmitted through the axis-cylinder but "jumps" over this medullated region of the nerve-fiber. And, since the electric current is the only possible physico-chemical process which could cause such a jump over the ridges, we are led to conclude further that the restimulating agent which excites the adjacent resting region of the nerve-fiber must be the action current developed at the active region.

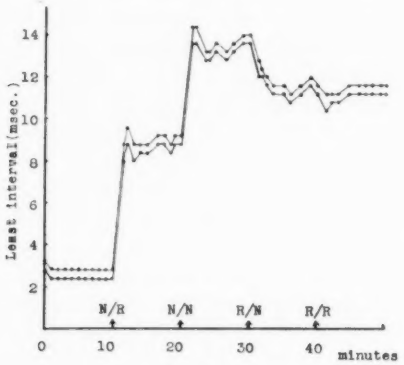

Fig. 5

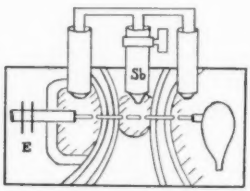

Fig. 6

Fig. 5. A similar experiment done with a 1.0 per cent cocaine solution. The narcotic was introduced first into the proximal pool.

Fig. 6. Arrangement to show the effect of the electrical resistance of the surrounding medium upon nervous transmission. Two nodes in the middle pool were rendered inexcitable with the narcotic, and conduction was thus abolished. Disconnection of the part Sb of the salt-bridge with the middle pool restored the conductivity.

Again, the objection may be raised that the medullated region of the axis-cylinder may transmit impulses of varying intensity. However, if conduction is assumed to be continuous and if the nodes are assumed to behave in an all-or-none manner, it would be impossible to explain the experimental fact that a nerve impulse cannot travel beyond three inexcitable nodes even when it can pass beyond two. And, since transmission is blocked only when regions including nodes are narcotized, we must assume either that the impulse jumps or that it undergoes a discontinuous steplike "decrement" as it passes beyond each narcotized node. This latter type of continuous transmission would resemble very closely

the discontinuous jumping, because the electric current flowing along the axis-cylinder would show an attenuation of exactly the same type in each case. At first it seemed impossible to distinguish these two possibilities experimentally, but more direct evidence for electrical transmission was obtained in a subsequent experiment.

3. The effect of the electrical resistance of the medium upon nervous transmission. If the nerve impulse jumps from one node to another by its action current, it should be theoretically possible to block or facilitate nervous transmission by changing the electrical resistance of the surrounding medium. And, conversely, if the effect of electrical resistance of the medium is proved to exist, we may now definitely conclude that the transmission of the impulse is electrical. This experiment was accomplished by using ridge-insulators. With a set of newly prepared ridge-insulators, it is easy to make the external resistance between two neighboring nodes enormous,—over 100 megohms. Since the internal resistance between two nodes is considered to be of the order of 50 megohms, ridge-insulators may be expected to influence the electrical transmission from node to node.

The method consisted in changing the leakage of current through inexcitable nodes. Figure 6 illustrates the experimental arrangements. Two sets of ridge-insulators were carefully coated with fresh paraffin before every experiment. Around each insulator, a salt bridge (consisting of three glass tubes filled with gelatin-Ringer gel and connected together with cotton wool moistened with Ringer) was placed. Two nodes of Ranvier were placed in the middle pool, and the fluid in this pool was replaced with a small amount of 4.0 per cent urethane Ringer solution. This often led to suspension of transmission. In case the transmission through the narcotized region was not suspended, a dilute (0.5 per cent) urethane solution was applied to the nerve in the other pools. When the transmission was completely blocked by narcosis, the middle glass tube (*Sb* in fig. 6) was elevated and thus the middle pool was electrically insulated from the other pools. It was found that transmission through the narcotized region was always restored by this procedure. When the end of the middle tube was again dipped into the fluid, transmission was immediately blocked. The experiment was simple and was consistently repeatable for hours.

This experiment is very easy to perform, probably the easiest of all the experiments described in this paper. As I shall show elsewhere, an irregular distribution of nodes as shown in figure 6 is very common among the toad's motor fibers. Further, it is easier to introduce into the middle pool two neighboring nodes (situated a short distance apart) than a single node. In narcosis of a single node, it is seldom that the transmission is blocked; but in this case, when the least interval for muscular summation is prolonged by narcosis, it can easily be observed that the least interval is

unmistakably shortened by removal of the middle tube. Removal of the whole bridge makes the excitability of the nerve-fiber extremely unstable and the muscle frequently shows powerful tetanic contractions, probably excited by atmospheric electricity.

This experiment is apparently comparable to that done by Osterhout and Hill (1929) on the large plant cell and can be regarded as conclusive evidence for electrical transmission in the nerve. Insulation of the inexcitable nodes reduces the leakage of the action current through these nodes and makes them behave as if they were covered with myelin sheath.

4. Summation and inhibition of the outflow of action current with subthreshold induction shocks. A nerve impulse passes beyond an inexcitable

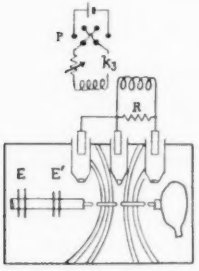

Fig. 7

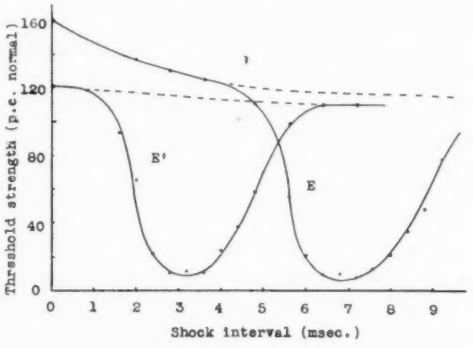

Fig. 8

Fig. 7. Arrangement for the experiments of figures 8 and 9. *P:* Pohl's reverser, k_3: contact of a Helmholtz pendulum, *R:* 6,000 ohms. Inductorium was coreless.

Fig. 8. Depression of the threshold by a blocked impulse. *E*: conditioning shocks were applied at about 4.2 cm. proximal to the operated region. *E'*: conditioning shocks at about 2 cm. proximal. The least interval was about 24 msec. and the intervals between shocks applied at the proximal electrodes were shorter than the least interval at that moment by 0.6 msec. Temperature, 5.5°C.

node when its accompanying action current is sufficiently strong to excite the irritable node beyond, and it fails to do so when the strength of the current is below the threshold. An impulse which is barely blocked by narcosis should therefore reduce the threshold of the node beyond the narcotized region. Hodgkin (1938) has studied this type of reduction of threshold with cold and compression blocks and has shown that the change in excitability occurs parallel to the change in the action current. I have also investigated this effect with the structure of the myelinated nerve-fiber in mind.

The arrangement shown in figure 7 was used. A narcotizing solution (2.5 per cent urethane Ringer) was introduced into the middle pool.

When the least interval for muscular summation was not sufficiently prolonged by this procedure, a dilute narcotic (below 1 per cent in urethane) was applied to the nodes in the proximal pool. Two stimuli were delivered to the nerve-fiber through the central electrodes (E or E' in fig. 7) at an interval just shorter than the least interval for muscular summation; and, at varying intervals after the second shock, the strength of a third (subthreshold) stimulus necessary to excite the node N_1 beyond the narcotized region (producing a summated muscular contraction) was determined with the exciting circuit shown in figure 7. By this method, I could demonstrate a distinct change in the threshold produced by the impulse which just failed to pass the block.

Figure 8 shows an example of the results. In this figure the threshold strength (as per cent of the normal) necessary to excite the node N_1 is plotted against the interval from the second induction shock applied at E or E'. The broken lines in the figure indicate the threshold level when the second shock was not applied; these lines deviate from 100 per cent due to the refractoriness produced by the first impulse. It is evident that, for a certain period after the arrival of the second impulse at the block, there is a marked depression in electrical threshold. The depression in threshold can be made as much as 98 per cent or more by controlling the interval between the shocks given at the central electrodes and by adjusting it to be just shorter than the least interval for muscular summation at that moment.

If the interval between the shocks applied at the central electrodes is made slightly longer than the least interval, the impulse set up by the second shock will just excite the node N_1. This excitation was found to be easily inhibited by superimposing a weak ascending current at an appropriate interval. The strength of the superimposed current necessary to inhibit increased, as was expected, with the increasing interval between the two impulses set up in the nerve fiber.

In the experiment shown in figure 9, a single stimulus was sent into the fiber at E or E' and the response was inhibited with ascending current pulses applied across the ridge. In this experiment the middle node was made inexcitable with a 2.5 per cent urethane solution as before, but the other nodes were all normal. When the interval between the two shocks was adequate, ascending currents of 3 to 4 times the threshold were sufficient to block the impulse.

Now, since we have clearly seen that nervous transmission depends upon excitation by the action current, it is important to know at what part of the nerve fiber the action current is developed. A nerve impulse set up at a node is transmitted, as is well known, in both directions, centrifugally and centripetally. In order that an electromotive force developed at a node may produce outwardly directed potential-drops at the neighboring

nodes on both sides, an electric double layer with inwardly directed moment must appear on the surface of the axis-cylinder at the node. Thus, it is clear that *the plasma membrane at the node of Ranvier is the place where the action potential is developed.* This is exactly what is expected from the membrane theory.

5. The electric circuit in the nerve fiber and quadripolar stimulation. In a previous paper (Tasaki, 1939b) it has been shown that, if a potential difference is applied between two neighboring nodes, potential-drops are produced at the nodes all along the fiber due to the insulating property of the myelin sheath and the relatively high resistance through the plasma membrane at the node. The resistance through the axis-cylinder (R in

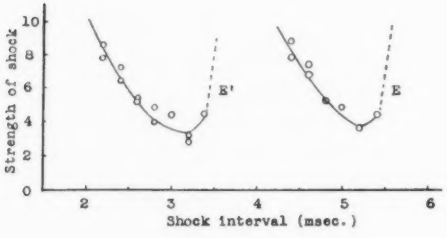

Fig. 9

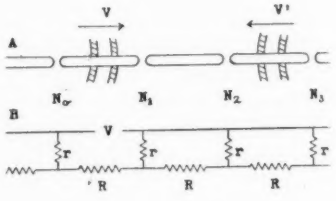

Fig. 10

Fig. 9. Inhibition of nervous transmission beyond an inexcitable node with an ascending induction shock. Ordinate: strength of ascending current necessary to block transmission (threshold for descending current as the unit). Abscissa: interval between the first and second shocks. E: the first shock applied at 59 mm. proximal to the isolated region. E': the first shock at 21 mm. proximal. Temperature, 5.5°C.

Fig. 10. A. Quadripolar stimulation of a single nerve-fiber. B. The electrical circuit in the nerve fiber. R: resistance through the internodal axis cylinder. r: total resistance of a node to the radial current. Resistance through the surrounding medium and the electric double layer at the plasma membrane are omitted in the diagram.

fig. 10) is considered to be purely ohmic and that through the plasma membrane (r) is at least partly reactive. If, for simplicity, all the R's are assumed to be the same and all the r's to be the same and all purely ohmic, it is predicted from the electrical network theory that the law of attenuation of the current flowing along the fiber is exponential, the potential drop through the nth node in figure 10B being given by

$$V_n = VB^n/(1 + B),$$

where V is the potential difference applied between the nodes N_0 and N_1, and B is the smaller root of the characteristic equation

$$B_2 - (2 + R/r)\, B + 1 = 0$$

(cf. Jeans, 1933, p. 319). This theory can be tested experimentally

by the method of multipolar stimulation, as these potential-drops produce excitatory effects.

Suppose that, in the quadripolar arrangements shown in figure 10A, potential differences V and V' are adjusted to constitute threshold stimuli and that excitation is to occur at the node N_2. In this case the resultant potential-drop through the plasma membrane at the node N_2 must always be constant, as we have assumed the plasma membrane to be non-reactive; hence we have

$$VB^2/(1 + B) + V'B/(1 + B) = \text{constant}.$$

As B is constant in a given nerve-fiber, this equation represents a straight line of which the slope is given by the value of B. In an entirely analogous manner, if excitation is assumed to occur at the node N_3, we have

$$VB^3/(1 + B) - V'B/(1 + B) = \text{constant}.$$

This also represents a straight line of which the slope is given by B^2. Thus the quadripolar stimulation of a nerve fiber is expected to yield a "tetragon" of which two sides in the first quadrant intersect the axes at arctan B and the remaining two sides at arctan B^2. It will easily be noticed that this "B" is nothing but what we have proposed to take as an index of polarizability from the experiments on tripolar stimulation.

Figure 11 is an example of the results of quadripolar stimulation. It is obvious that the results lie on straight lines which constitute a "tetragon". The values of $\cot\beta'$ and $\cot\beta''$, when measured with constant current pulses of a duration of 0.5 msec., were generally 0.30 to 0.55; and the values of $\cot\gamma'$ and $\cot\gamma''$ were between 0.07 and 0.20 and were a little smaller than the square of $\cot\beta'$. This discrepancy from the simple electrical network theory is considered to be due mainly to our ignoring the capacity of the plasma membrane. Moreover, the extreme irregularity in the internodal distance seems to complicate further such a mathematical treatment of the problem.

There is little doubt that the above-stated rough estimation of the spread of stimulating currents along the fiber holds for the spread of the action current. If an electromotive force V is developed at the plasma membrane at the node N_0 in figure 10B, it will easily be found that the potential-drop produced at the nth node is given by the formula

$$V_n = VB^n.$$

It is interesting that the action potential developed at the node N_0 is just $(1 + B)$ times as effective as the external electromotive force of the same magnitude and duration applied between the nodes N_0 and N_1.

It would probably be possible to establish a more complete physical theory as to the electrical network in the nerve-fiber. But, as we know nothing definite about the physical nature of the excitatory state, it would be difficult to make it available for the theory of nervous transmission. That some process besides polarization plays a part in electric excitation

would be evident from the experimental fact that narcosis changes the chronaxie but not the polarizability.

6. The general theory of nervous transmission and the interpretation of various phenomena. Lillie in 1925 first suggested the possibility of "electro-saltatory" transmission of the nerve impulse in the myelinated nerve fiber. Lillie observed that, when a passive iron wire is covered with a glass tube broken at regular intervals, the activation does not spread over the whole wire but jumps from one break to another; and from this observation he assumed a similar jump of the nerve impulse from one node of Ranvier to another. Several papers that have been published since assume and discuss electrical transmission of this type (Gerard, 1931; Rashevsky, 1933 and 1934; Zottermann, 1937). Now the hypothesis is

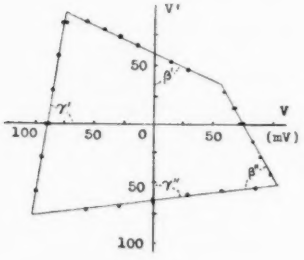

Fig. 11

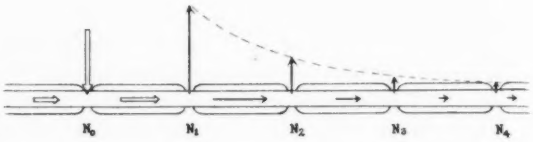

Fig. 12

Fig. 11. A "tetragon" obtained by the quadripolar stimulation of a nerve-fiber. The duration of the rectangular current pulses employed for stimulation was 0.5 msec. Temperature, 8°C.

Fig. 12. Distribution of the outwardly directed action-current flow at the boundary of the active region of a nerve fiber.

provided with a series of rigid experimental proofs and is further extended to explain various phenomena in nervous transmission.

Figure 12 illustrates the flow of the action current at the boundary of the active region. The resting nodes are excited with the sum of "action-current outflows" caused by all the nodes in action. Even when several nodes, say N_1 and N_2 in the figure, are rendered inexcitable, the current flowing outward through the irritable node N_3 is, in most cases, strong enough to excite it. As Hodgkin (1938) states, there is a wide margin of safety in the electrical transmission of impulses.

Let us introduce, modifying Hodgkin's idea, a new quantity, the "*safety factor*" *in nervous transmission*, as indicating the ratio of the total action-current outflow through a node to the current (of the same shape) required for excitation. When this factor is larger than a unity, the node is excited before the total current is used up and transmission occurs. When it is larger than about 2.5, the impulse has the ability to jump over one in-

excitable node, for the current decreases to from 0.3 to 0.5 (index of polarizability) times the original value when it spreads beyond a node. In freshly excised nerve-fibers, impulses jump over at least two completely inexcitable nodes. Therefore, the safety factor in the normal nerve-fiber is greater than about 8; it is probably about 10 and seems to decrease gradually with the time after isolation of the nerve-fiber.

The main causes which change the safety factor are probably as follows:

1. The magnitude and duration of the action potential developed at the plasma membrane.
2. The irritability of the node.
3. The polarizability of the plasma membrane.
4. The true latency of response.

A decrease in these quantities which characterize the nerve-fiber will decrease the safety factor in transmission. It seems at present impossible to formulate theoretically the accurate relation between these quantities and the safety factor, due to lack of our knowledge of the excitatory state and the latency. But, if we adopt the simple network theory stated above and if we assume the transmission to be sufficiently rapid, the safety factor (s.f.) will roughly be given by

$$\text{s.f.} = \frac{VB}{1 - B} \div \frac{EB}{1 + B},$$

where V is the action potential developed at the membrane, E is the threshold voltage measured with a current pulse of the same temporal configuration as V, and B is the polarizability measured with the same current pulse. The first term $VB/(1 - B)$ expresses the total action-current outflow through a node to be excited, and the other term $EB/(1 + B)$ represents the current necessary to excite a node. Since E and B are about 30 millivolts and 0.5 respectively, the magnitude of the action potential in the normal nerve-fiber should be of the order of 100 millivolts. This estimate must of course be regarded with great caution.

A practical method of measuring the safety factor is supplied by the experiment shown in figure 9. If an induction shock of the strength of n times the threshold is required for inhibition of transmission, the safety factor in the jump of impulse beyond a single inexcitable node should be equal to $(n + 1)$, as it was shown in a few preliminary experiments that the law of superposition holds good in such cases. Further, the safety factor in the normal nerve-fiber can be obtained by measuring the polarizability of the inexcitable membrane.

The first problem which I want to explain in terms of the elementary properties of the nerve-fiber revealed by the present investigation is that of the "decrement" of the impulse in the narcotized region. Adrian (1912) and many others have shown that in order to extinguish conduction by

local anesthesia of a nerve-trunk, the narcotic had to be applied for a longer time, the shorter the length of nerve upon which the narcotic acted. Further, Kato (1924) found that this effect of the length of the narcotized stretch is limited to about 6 mm. and consequently that a long stretch can resist narcosis for the same length of time as a short one provided the latter is longer than this "limit length." All these experimental facts can clearly be interpreted in terms of the "jump" of impulse beyond a short narcotized stretch. As we have already seen, a normal impulse is capable of jumping over about two inexcitable nodes; and, since the internodal distance can be 2 to 3 mm., most of Kato's limit length must be attributed to the length of nerve beyond which a normal impulse can jump by its action current.

Davis and his co-workers (1926) expressed the view that the transmission of the impulses along a (weakly) narcotized nerve could be "transitionally" decremental. As long as we define, as these authors do, a nerve impulse as a propagated tendency to excite the adjacent inactive region of nerve (which we found to be an electrical current), the intensity of impulse should show a transitional change at the boundary of the normal and weakly narcotized regions. Not only the intensity of impulse but also the conduction rate is considered to show transitional decrement and increment at the boundary. As regards the problem of decrement, we must agree in general with Davis *et al.*, except for their assumption that transmission is continuous in each fiber.

The next problem we are interested in is that of local non-conducted response. If the safety factor in the transmission is decreased to a low value (below one, but not equal to zero), a local response may occur at the seat of stimulation. This seems to be actually the case during the absolutely refractory period. In the experiments shown in figures 4 and 5, it can be seen that the least interval for muscular summation is not determined by the refractoriness of the narcotized node; in this case all the nodes which take part in the transmission are normal, and it is obvious that the least interval is determined by the interdependence of the excitability and the power of restimulation at the two regions on both sides of the narcotized region, namely, by the recovery of the safety factor. The least interval in normal and in uniformly narcotized nerve represents the length of the absolutely refractory period, and this also appears to be determined by the recovery of the safety factor in the transmission and not by the recovery of the local response.

[4] When the least interval of a node was measured with a tripolar arrangement (fig. 1, inset), it was found that the interval was lengthened not only by a weak narcosis of that particular node (N_0 in fig. 1) but also by narcotization of the adjacent node (N_1); and, as the least interval is always longer when both of these nodes are narcotized than when the same narcotic is applied to either one of them, it is clear that the impulse set up

at an early stage in the refractory period fails to be transmitted when the adjacent node is also narcotized. In the normal nerve, it is well known that the least interval changes with the direction of the currents employed for measurement, and this effect of the direction of the stimulating current is more marked in the narcotized nerve-fiber. From these data we may conclude that the recovery curve first introduced by Adrian and Lucas (1912) consists of two different parts, one, the absolutely refractory phase, representing the recovery of the safety factor in transmission and the other, the relatively refractory phase, representing the recovery of local excitability.

Recent work by Katz (1937) appears to suggest that a local response may also exist in a fresh normal nerve, but my own observation indicates that it is improbable. I have shown that the threshold of a node of Ranvier is independent of the irritability of the adjacent nodes. This seems to be convincing evidence that the nerve impulse is released by a trigger mechanism. A local response in a fresh normal nerve, if it exists at all, has practically no power restimulation.

Lastly, let us turn our attention to the effect of repetitive stimulation of a locally narcotized nerve-fiber. We have already seen that, when two impulses are sent along a nerve-fiber to face a stretch including a small number of inexcitable nodes, the second impulse may fail to be transmitted beyond the narcotized region unless it follows the first at an interval longer than a certain critical value (i.e., the least interval). This indicates that the safety factor in the jump of the second impulse is smaller than that of the first. Refractoriness of the node just beyond the narcotized region and reduction of the action potential and polarizability during the refractory period seem to be the only possible causes for this reduction of the safety factor. If the second impulse fails to be transmitted, the excitability of the node beyond the narcotized region should recover to normal, but the action-current outflow which stimulates this node may still be subnormal. Thus, when the safety factor in the jump of the first impulse is slightly greater than unity, all the successive impulses except the first may fail to pass beyond the narcotized region. We may therefore conclude that the apparent inhibition of successive impulses (Wedensky's inhibition) is due to decrease in the ability of the proximal (normal or weakly narcotized) region of nerve to excite a node beyond the region of impaired conductivity. A few additional experiments revealed that reduction of polarizability during the refractory period is only slight in the normal nerve fiber, but the action potential is known to suffer a considerable reduction (Gasser and Erlanger, 1925). Undoubtedly the safety factor in the normal nerve fiber is decreased by repetitive stimulation.

Discussion. The idea that the nerve impulse may jump beyond a stretch of impaired conductivity is by no means new. Werigo (1899) long

ago believed that a wave of excitation was capable of setting up a similar disturbance in a normal region beyond the impaired stretch. Then this idea was abandoned and for many years was replaced by the theory of continuous decrement. Quite recently, this old idea of jump was revived by Osterhout and Hill (1929) in their beautiful work on the plant cell. Now, the presence of jump in the myelinated nerve fiber is, I believe, firmly proved by a number of rigid experiments. The only reasonable objection against this conclusion seems to be my own observation that cooling the medullated region of a nerve-fiber blocks conduction (cf. Kato, 1936), but this objection may be eliminated by assuming demarcation surfaces of very high electric resistance at the boundaries of the frozen protoplasm.

SUMMARY

1. A nerve impulse can pass beyond a few completely inexcitable nodes of Ranvier.

2. The least interval for muscular summation increases as the number of inexcitable nodes over which impulses must jump increases.

3. It is possible to block or facilitate nervous transmission by changing the electrical resistance of the surrounding medium. It is concluded that nervous transmission depends upon stimulation of the resting region of the nerve-fiber by the action current developed at the active nodes, and that the impulse jumps, as Lillie suggests, from one node of Ranvier to another.

4. Hodgkin's experiment showing a depression of threshold by a blocked impulse was reëxamined on the isolated single nerve-fiber with positive results. Furthermore, transmission beyond an inexcitable node can be blocked by a weak ascending induction shock.

5. The electric circuit in the nerve-fiber has been investigated by the method of quadripolar stimulation.

6. Nervous transmission through a narcotized region of nerve is discussed and various phenomena are interpreted in terms of the "safety factor" in nervous transmission.

7. The nerve impulse undergoes transitional decrement and increment as it passes through a narcotized region.

8. The importance of the plasma membrane at the node of Ranvier in excitation and transmission is stressed.

It is a pleasure to express my deep gratitude to Dr. H. Davis for encouragement and assistance in preparing this paper. I also wish to thank Prof. G. Kato and Dr. T. Hayashi for their valuable advice.

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THE RESPIRATORY AND CIRCULATORY RESPONSES TO INTRAVENOUS OXYGEN AND THEIR RELATION TO ANOXEMIA

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Received for publication March 22, 1939

In 1927 Bourne and Smith (1) reviewed the experimental work on intravenous oxygen and used this procedure in attempting to relieve anoxemia produced previously by lowering the oxygen tension of the inspired air. Although the amounts given were theoretically sufficient to supply enough oxygen to overcome the deficiency then existing, they found that the anoxemia was accentuated and that this effect was greater in the more severe grades of anoxemia. Under the conditions of the experiment the arterial blood passed through the lungs where it had ample opportunity to come into equilibrium with the alveolar air but failed to do so.

They also noted transient attacks of dyspnea during the injections. It is well known from the work of Binger (2) that rapid shallow breathing occurs when multiple emboli of the pulmonary arterioles and capillaries are produced experimentally in dogs. The object of this investigation was to study the anoxemia and the associated respiratory and circulatory changes. Since previous work had demonstrated that anoxemia cannot be prevented or relieved by intravenous oxygen, no attempt was made to produce anoxemia before the injections of the gas. All experiments were done on animals breathing air or pure oxygen.

EXPERIMENTAL. Dogs were anesthetized with morphine sulfate and sodium barbital. The carotid blood pressure and the respirations were recorded. Oxygen was introduced into the femoral vein through a cannula connected with two large syringes. A two-way stop cock in the circuit with one of its passages leading to the oxygen tank permitted easy and rapid refilling of one syringe while the contents of the other were being discharged into the vein. Samples of arterial blood were collected from a femoral artery through a blunted needle long enough to reach the bifurcation of the abdominal aorta and thus minimize the effects of stasis during sampling. The blood was analyzed for oxygen by the method of Van Slyke.

RESULTS. The amounts of oxygen that can be given lie within the limits already known. Two to 3 cc. per kilogram of body weight per minute is the average that can be maintained with safety although much larger amounts can be given for short periods.

The depression of the arterial oxygen content as a result of the giving of 3 cc. per kilo per minute to six dogs breathing air at atmospheric pressure is shown in table 1. The depressions are expressed as per cents of the values before the injections. The samples were taken during the third minute of injection. It is clear that intravenous oxygen not only cannot relieve a previously existing anoxemia but will induce anoxemia when none is present.

Blood pressure and respiration. The factor limiting the rate of injection is a sudden fall of arterial blood pressure. However, it is possible to give the gas at such a slow rate that there is no fall of pressure in the systemic circulation, while the arterial oxygen falls with each injection. The effect

TABLE 1

ANIMAL	ARTERIAL OXYGEN CONTENT EXPRESSED AS PER CENT OF VALUE BEFORE INJECTIONS			
	1st injection	2nd injection	3rd injection	4th injection
1	94	80		
2	91	92	91	85
3	90	90	91	84
4	89	87		
5	86	86	75	
6	84	84	72	

on the respiration is more striking and more constant. An abrupt increase in rate occurs and this persists after the oxygen injection has been stopped until long after the arterial oxygen content has returned to normal. Section of the vagi prevents the increase of respiratory rate. Increasing the oxygen tension of the inspired air does not, but in each case the arterial oxygen content falls as before.

Time of fall of arterial oxygen content. Analysis of samples taken one minute after the beginning of the injection demonstrated that the fall of arterial oxygen took place as soon as this. In order to determine the time of fall more accurately a procedure described by Kramer (3) was adopted. He has shown that the amount of light transmitted by blood flowing in the large vessels is inversely proportional to its saturation with oxygen. A chamber was designed to fit closely about the carotid artery but permitting the blood to flow through freely. A small bulb furnished light which passed through the arterial walls and the flowing blood to reach a narrow slit on the opposite side. Behind this slit a photoelectric cell was placed. The deflections of a string galvanometer

recorded the variations of current in the cell. These indicated decreased arterial saturation, beginning within 10 to 15 seconds after the start of the injection.

The pressure in the right ventricle. As seen, oxygen can be given at such a rate that there is little or no effect on the arterial blood pressure. Further to investigate the events taking place during injection a cannula was put into the right ventricle through the internal jugular vein and another into the carotid artery. Under these conditions this pressure in the right ventricle recorded optically by the Wiggers method (4) rises at once when oxygen is introduced and remains elevated until it has been absorbed as indicated by the disappearance of the rustle audible over the precordium.

Discussion. Meakins and Davies (5) have shown that anoxemia may result from imperfect pulmonary ventilation due to rapid shallow breathing. The anoxemia described here cannot be due to this since it comes on before the respiratory change and disappears much sooner. Moreover section of the vagi which prevents respiratory changes does not prevent the anoxemia. The imperfect aeration of blood in its passage through the lungs is therefore probably not due to a decrease in the oxygen tension of alveolar air.

A rise in right ventricular pressure invariably accompanies the anoxemia, beginning at almost the same time. This points to a disturbance in the pulmonary circulation as the cause of the rise of pressure. Presumably the blocking of a number of channels by gaseous emboli forces a greater quantity of blood to traverse fewer channels, the absence of fall of aortic pressure indicating that the total amount of blood reaching the left ventricle is unaltered. Hence it might be argued that the more rapid passage of blood through the channels still open would not leave time for complete oxidation at the normal rate of diffusion of oxygen through the alveolar walls. Raising the diffusion rate, however, by increasing the oxygen tension of inspired air had no effect on the anoxemia and this argument is therefore invalid. On the other hand it does not seem probable that the presence of gaseous oxygen in the lung capillaries would alter their permeability to oxygen. It should be noted in this connection that the oxygen consumption of the animal remained unaltered, the oxygen introduced by vein being compensated for by a decreased absorption from the lung. It is, however, clear that the disturbance in the pulmonary circulation is in some way connected with the anoxemia.

With regard to the increased respiratory rate, these experiments show that it is of reflex origin, the afferent path being by way of the vagus. It is not due to the anoxemia. This may be compared with Binger's observation that the tachypnea seen in patients recovering from pneumonia is rarely altered by an oxygen tent while the anoxemia is completely relieved. In this case, also, a reflex arising from a disturbed pulmonary circulation is probably the cause.

SUMMARY

1. The temporary pulmonary embolism produced by intravenous oxygen is accompanied by anoxemia beginning almost immediately. This is related to increased pressure in the right ventricle while the aortic pressure, if the injection is sufficiently slow, may not fall. The causes of this anoxemia are discussed.

2. There is a marked increase in pulmonary ventilation beginning somewhat later than the anoxemia and persisting long after it has disappeared. This is due to a reflex passing up the vagus probably arising from the pulmonary circuit.

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A STUDY OF THE FUNCTIONAL SIGNIFICANCE OF THE CEREBELLAR AND DECUSSATING MEDULLARY PATHWAYS OF THE FROG

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Received for publication May 2, 1939

Gaupp, Kappers, Röthig, Larsell, Coghill and Herrick have contributed greatly to our knowledge of the morphology of the amphibian brain. From the terminal sensory nuclei there are, in this phylum, long fiber systems which are extensive but poorly organized (Herrick, 1930), spinal and bulbar lemnisci, but no exact anlagen to the medial lemnisci, and reticular nuclei which are not as well organized as in other phyla (Kappers, 1936). Herrick's (1933) study of the ascending fibers in Necturus indicates that "each lemniscus tract shows an incipient but incomplete specialization in relation to a particular system of receptors and afferent nerves" and that a single fiber of these secondary neurons may divide and follow each of three different courses. He believes that one sensory system which activates the lemniscus system may have some measure of dominance over the others and that, as we advance in the animal kingdom, the dominance increases. These conclusions are "inferences drawn from anatomical arrangements, and there is as yet little experimental proof."

These morphological facts, which would lead one to suspect that lesions of the lemnisci in the amphibian medulla would not give rise to such marked effects as in higher forms, have formed the basis for this experimental study. The work was confined to an attempt to determine, if possible, the effect of severing the decussating paths of the medulla on the behavior of the animal. The work on the cerebellum grew out of an attempt to eliminate the effects of lesions of afferent and efferent paths of this structure in the interpretation of the results.

The general procedure was to produce anatomical lesions in anesthetized frogs, working under a binocular microscope. Serial sections were made of the brains of 55 of the 250 frogs operated upon. The Pal-Weigert, Marchi, and celloidin modification of the Weil stain were used.

METHOD OF EXAMINATION OF ANIMALS. The following observations were made at intervals of one to two days:

[1] I am deeply indebted to Prof. F. H. Pike for suggesting this problem and for his constant guidance and encouragement.

1. The spontaneous jump, its direction, number of movements, vigor, speed of flexion and extension, landing posture.

2. The jump induced by visual, light tactile or deep pressure stimulation. The points noted as in 1, when a violent movement was made.

3. Swimming movements, type, direction, vigor, use of limbs, orientation in water; righting movements, their speed and direction.

4. Posture, color, respiratory rate, pupils when the frog was found undisturbed in the bowl.

5. Tonus, observed in unilateral lesions, in previous observations, and also by holding the frog under the forearms and noticing any differences in length or position of the legs.

The following tests were also employed:

1. Performance tests. The animals were made to jump from water of various depths to land; to jump from vessels with walls of different heights and slopes; to avoid obstacles placed suddenly in the path; to jump from various heights to the ground.

2. Equilibrium tests. These involved response to forward, backward, and lateral acceleration, to tilting in all directions, and to rotation, with the animals in various positions on the wheel (Tait and McNally, 1925). Each group studied was compared with controls. Only the deficiences of the experimental animals are mentioned.

EXPERIMENTAL WORK ON THE CEREBELLUM. Herrick (1924) with whom Larsell (1925, 1929, 1937) agrees, believes that the amphibian cerebellum develops under two distinct influences of *a*, the spino-cerebellar tracts affecting the corpus cerebelli, and *b*, the vestibular and lateral line pathways, affecting the auricular lobe. In the frog, the auricular lobe would then be concerned with the reception and elaboration of vestibular impulses.

Microscopic examination of the brains of frogs in which the cerebellum was removed, or bilaterally severed, showed that in no case was the auricular lobe completely removed. The cut was either through this region, or at the lateral border of the corpus cerebelli. It is safe to say that by this type lesion, the spino-cerebellar fibers, either at their entrance into the cerebellum or at their termination in the granular layer, are severed.

Examination of twenty-one frogs, in which the cerebellum was removed, indicated that severing the spino-cerebellar fibers produced little variation in ordinary behavior. In the more exacting performance tests, there were slight deficiences. When the animals jumped from a height of two feet, they landed with less spring than a normal frog, and the legs tended to spread. There was some difficulty in jumping out of a container with perpendicular sides six and a half inches high, and an inside area so small the the landing place could not be seen from within. In this case, the landing was heavier than normal, and the landing position flatter, with

the legs slightly spread. The animals quickly "drew themselves together" and assumed the normal posture. Mayer and Heldfund noted only slight dysmetria upon cerebellar extirpation in frogs.

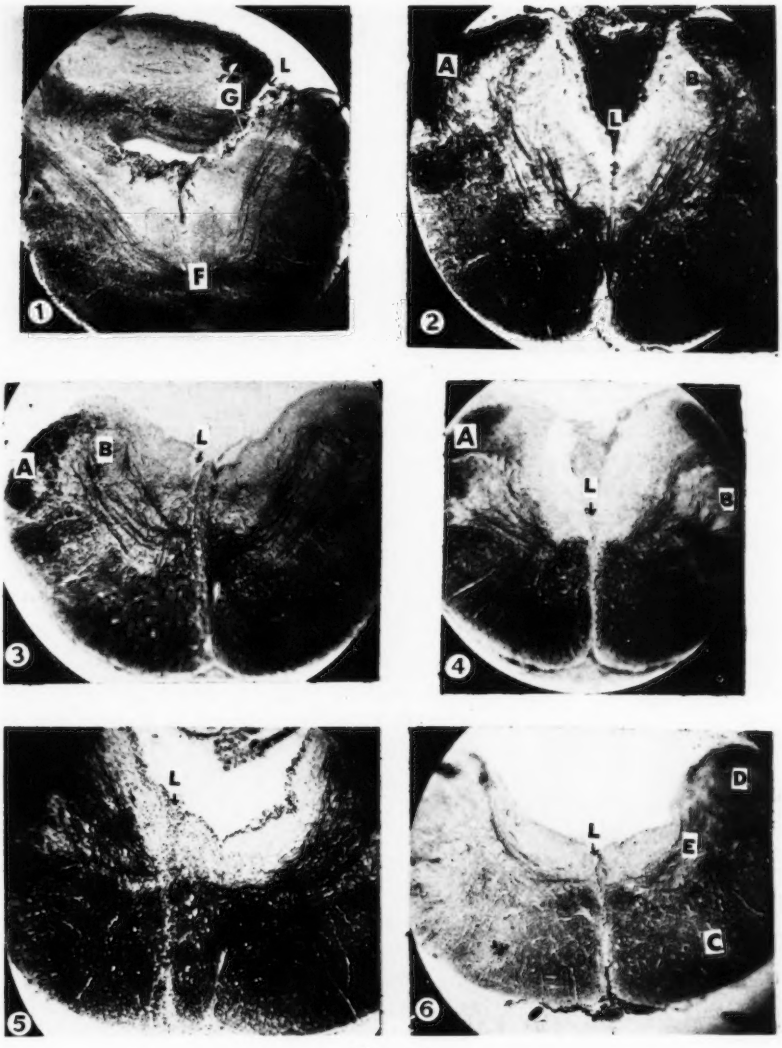
In unilateral lesions of the cerebellum (fig. 1) with complete lateral incision on one side, and no other injury to the brain, the behavior of the animal differed in no significant way from that of the control until the fourth day. The slight deficiency in the more accurate performance tests was noticeable in all ten animals of this series, but was not confined to one side. During the first week, the legs began to spread, and convulsions, with no difference in the degree of extension of the limbs on the two sides, appeared a few days later. Mayer and Heldfund noted more severe disturbances upon unilateral extirpation, but the ventral extent of their lesions was not mentioned. Lutterotti reports more severe disturbances in Hyla, which she attributes to the greater development in this genus.

A medial longitudinal incision of the midbrain was made in ten frogs in order to sever the decussating fibers of the brachium conjunctivum. Serial sections of the brains of two showed that, in one of them, the cut extended to the caudal limits of the mesencephalon, severing completely the commissura ansulata, in which the fibers of the brachium are said to decussate. There was no significant change in behavior of any of the frogs of this series. Longitudinal lesions of the rostral medulla would sever the decussating component of the tractus cerebello-motorius (Röthig, 1927). Except for a tendency to walk rather than jump when first released, a trait noticed in other frogs in which the median incision in the medulla was not this far rostral, no abnormality was noted.

We can conclude, from the above reports, that severing the spino-cerebellar fibers bi- or unilaterally within the corpus cerebelli produces a slight deficiency in the performance of acts demanding precision and accuracy. The severing of the decussating fibers of the brachium conjunctivum and tractus cerebello-motorius produces no apparent abnormality in the action of the frog.

Marchi preparations after removal of the corpus cerebelli showed a degeneration in the ventral funiculus at the level of the closed medulla, and extending down into the thoracic region of the spinal cord. The degeneration is scattered through the medial longitudinal fasciculus in the caudal portion of the medulla. It was not marked in the rostral medulla.

In twenty out of the thirty-one animals, in which there was a bi- or unilateral severing of the cerebellum, actual convulsions, or strongly convulsive movements appeared. Of the other eleven, nine showed exaggerated spreading of the legs, which is the behavior which usually preceded the convulsions, and only two showed no sign of increased extensor tone when incited to jump. The spreading of the legs, which was a lateral thrust, first appeared upon vigorous stimulation, and then, a day or so later,



Figs. 1–6

Key to photographs: *A*. Tracts of the Vth and VIIIth nerves. *B*. Fasciculus solitarius and nucleus. *C*. Superior olive. *D*. Dorsal nucleus of VIIIth nerve. *E*. Lateral lemniscus. *F*. Tractus cerebellaris motorius cruciatus. *G*. Cerebellum. *L*. Lesion.

Fig. 1. Cross section brain stem level of cerebellum. Unilateral lesion of cerebellum. Weil stain.

Fig. 2. Cross section of brain stem level of caudal medulla. Central longitudinal lesion of caudal medulla shown. Respiration not resumed. Pal-Weigert stain.

Fig. 3. Cross section of brain stem level of caudal medulla, rostral fibers of twelfth nerve shown. Caudal end of longitudinal lesion left of central sulcus shown. Pal-Weigert stain.

Fig. 4. Cross section of brain stem low mid-medulla. Caudal end of central longitudinal lesion shown. Pal-Weigert stain.

Fig. 5. Cross section of brain stem level low mid-medulla. Caudal end of longitudinal lesion lateral to central sulcus shown. Pal-Weigert stain.

Fig. 6. Cross section brain stem level rostral medulla; caudal end of longitudinal lesion shown. Weil stain.

in the spontaneous jump. It appeared anywhere from the second to the sixteenth day after operation, and most frequently at the end of the first week after operation. The actual convulsions might occur as early as the second day after operation, if the animal was in poor condition; they usually occurred during the second week. The longest time between the operation and convulsions in the cerebellar animals was eighteen days. The convulsions were initiated by cold water or an effort to jump. The animal became suddenly rigid, legs markedly extended, forearms crossed over chest, or extended, the eyes drawn in, the back usually concave dorsally. The tonic convulsions lasted about a minute, except in the case of unilateral lesions, where the duration was only twenty to thirty seconds. This tonic phase passed into a clonic phase, which lasted another minute or two, and this was followed by a period of complete relaxation, lasting two to three minutes.

Convulsions occurred in six frogs in which median longitudinal lesions of the medulla were made. In one, the convulsions lasted one day and disappeared. Microscopic examination showed that, in three of these frogs, there was some damage to the cerebellum; one brain could not be sectioned; in another a clot was found impinging on the cerebellum; in the sixth, microscopic examination revealed no damage to the cerebellum.

The reason for these convulsions is not obvious, and none of the numerous hypotheses proposed seems satisfactory. The fact that after excision of the cerebral hemispheres, the convulsions were only briefly intermitted, one to three days, does not support Lapinsky's view (1899) that the cerebrum is necessary for the genesis of these convulsions of bulbar origin. While increased extensor tone has been reported after excision of the cerebellum or its penduncles in mammals (Horsley, 1907; Theile, 1905; Hill, 1900; Pollack and Davis 1930; Magoun, Hare and Ranson, 1937) convulsions arising from tumors of the posterior fossa, while noted in a few cases (Jackson, 1906; Horsley, 1886) are considered rare (Kraus, 1929). Fulton (1938) states that "all recent writers on the cerebellum agree that the enhanced postural reflexes which follow complete ablation in dogs, cats, and birds are due specifically to interruption of the paleocerebellar projections from the anterior lobe."

Since, in some of my cases, convulsions have developed without direct lesions of the cerebellum, although most of the cases involved some cerebellar injury, I am not prepared to say that it is extirpation of the cerebellum alone which is responsible for the appearance of extensor tone and convulsion. I believe we may safely conclude, however, that the appearance of increased extensor tonus and subsequent convulsive seizure is a frequent secondary result of bi- or unilateral cerebellar lesions.

Experimental work on the medulla. For purposes of classification, the region of the medulla caudad to the emergence of the tenth nerve roots

in here called the caudal medulla; from that level to the superior olive, the mid-medulla; from the superior olive to the rostral border of the rhombencephalon, the rostral medulla.

The course of the internal and external arcuate fibers has been described by Röthig (1927), Holmes (1903), Larsell (1923), Kappers and Hammer (1919) and Gaupp (1889). Gaupp believes there is a functionally significant connection between the dorsal horn cells in the caudal medulla and the nuclei of the eighth nerves, through fibers in the external arcuates. Larsell and Röthig describe the tractus bulbo-cerebellaris as connecting primordial nuclei of gracilis and cuneatus with the vestibular portion of the cerebellum, also through the external arcuates. Herrick (1930, 1933) found that in Necturus, the spinal lemniscus, containing fibers from the cutaneous and proprioceptive organs, forms a synaptic connection in a dorsal nucleus in the cervical cord and crosses at the level of the calamus scriptorius, and one segment below.

An attempt was made in fifty-two frogs to cut any decussating fibers in the caudal medulla. The fatality in this group was the highest of all, probably because of the presence of the arteria basilaris (Gaupp) ventral to the central sulcus. In fifteen of the animals, there was no complete recovery, since no respiratory movements were reëstablished. There was, however, recovery from anesthesia, and some movements of the limbs. But the most constant result was a change in the character of the heart beat. The beat did not increase significantly in rate, but the force was so great that the action could be observed through the chest wall at a distance of several feet from the animal, for several hours after the operation. There was usually a vaso-constriction in the abdominal wall. The cut, in three animals of which serial sections were made, was straight and in the mid line. In two of the frogs, there was a small amount of blood on the ventral surface of the medulla, and one (fig. 2) showed some blood in the fourth ventricle. The caudal level of the cut was low in the medulla at a level where the fasciculus solitarius and its nucleus are still medially placed, but this was no lower than in nine others of this series, in which respiration was resumed. The cardiac response is probably to be viewed as a compensatory reaction to the impaired respiratory activity.

The twelve of these fifty-two frogs which lived long enough, and regained sufficient motor facility to test for functional deficiences, showed four changes of behavior (fig. 3). The first change noted was a rotary action which the frog exhibited. This is not to be confused with forced movements due to lesions in the lateral medulla, or other parts of the brain. It occurred in cases where the animal had a slight list to one side, and also where the animal was well poised. It was present during the whole post-operative life of the animal, but became less noticeable after the second week. The frog sat quietly on the floor after removal from its

bowl. In a short time, it turned its head slowly to one side or another, and then turned the whole body in the same direction. This action, at times, was continuous until the frog had rotated as many as eight times in one direction, or it was interrupted by long pauses, and only a partial revolution made in one direction. In some cases, this vacillating behavior continued for ten to fifteen minutes, and the animal never moved more than a foot from the original spot, although jumping or turning continually. After the rotation in one direction, the frog might repeat the performance in the other, or might jump in a perfectly straight line. In two frogs, the rotation was predominantly to one side. Histological examination revealed a lesion on the side of the caudal medulla opposite to that of rotation. The frogs made correct compensatory movements to tilting, rotation, and linear acceleration. Those with a list to one side did not react normally to tilting in that direction.

The second variation in behavior was that the frog was unable to maintain its balance, or to compensate correctly if the movement was sudden or extreme. The animal then lost orientation completely, and tumbled toward the side to which it was tilted. This deficiency was most obvious the first week after operation. McNally (1937) found that different parts of the vestibular apparatus are stimulated by slow and rapid tilting.

The third symptom was that, in the immediate postoperative period, the frogs were highly excitable and, when released, jumped so nearly vertically that they hit objects above them, and landed in almost the same place. They hit obstacles placed in their path, rather than jumping across them. This type of behavior became less obvious, but could be detected after a month. When the animal jumped from a height of two feet to the ground, it frequently fell on its head, and landed with feet spread out. When the frogs were placed in a small box with perpendicular sides six inches in height, they made many attempts to scale the wall, jumped into it as many as ten times and, if successful at all, jumped just high enough to reach the top, and toppled to the ground. Three of the animals, when placed in a bowl of water, jumped up instead of out in attempting to escape.

The fourth change noted was a difference in the flexion of the legs after the operation. In six animals, an incomplete flexion was noted in the spontaneous jump. In the others this was noted only when they were vigorously stimulated. The legs spread upon landing, and flexed slowly. In three frogs this was particularly noticeable on one side, and the symptoms increased in severity. A normal position of the leg could be maintained, but it was usually unflexed. Histological examination revealed a lesion to the same side of the f.l.m. as that of the incompletely flexed leg. The turning was usually to the side opposite to this leg.

The assignment of particular symptoms to lesions of particular tracts

is difficult because of the extensive intermingling of various types of fibers. The tracts most probably concerned are 1, that described by Gaupp; 2, the tractus bulbo-cerebellaris, or 3, the spinal lemniscus (Herrick) if it is in similar positions, in the frog and Necturus. The symptoms observed in frogs after lesions in this region approximate those observed in cats after lesions of the medial lemniscus (Pike, 1937).

Central longitudinal lesions were made in the mid-medulla of eleven frogs (fig. 4). In eight frogs there were no abnormalities that showed in normal jumping, swimming, posture or vestibular tests. Moving pictures were made of several of these animals, and again a more careful analysis of their reactions revealed no disturbances. Serial sections were made of the brains of five of these cases and the site of lesion confirmed. In one, in which there was a slight list to the left, the cut was found to impinge on the right f.l.m. The lesion was distinctly to the side in two frogs (fig. 5) and in these cases there was no list to either side, and no forced movements. A sideward flinging of the legs, which bore no resemblance to the lateral thrust present after cerebellar removal, was present in a few frogs, but was not a constant symptom. Microscopic examination of the brains of the three frogs which did not respond normally, revealed injury to the brain other than the central lesion.

A Marchi stain was done on the brains of two frogs of this group, which lived well over a month. The lesions in these frogs were to the right of the f.l.m., and the degeneration was predominantly on that side. The degeneration was present in the cervical region of the cord, and extended up to the lesion.

A study of the behavior of the two frogs, in which the lesion is far to the side so that it probably cuts more arcuate fibers from that side than the other, shows that in all tests applied, the reactions appeared normal.

Longitudinal lesions in the rostral medulla were made in twelve frogs (fig. 6). Except for those mentioned above, which became convulsive some time after operation, eleven of the twelve were essentially normal in their behavior. One was distinctly abnormal in posture, jump and movements of the limbs. Serial sections of the brain of this animal revealed a large clot pressing on the dorsal medulla. Since there was no difference in either the extent or the position of the lesion, as compared with the others of this group, I believe that the clot was the agent producing the atypical behavior.

I have used the term "essentially" normal because there were slight variations in the behavior of the animals. A few frogs flung their legs to the side, or crawled upon release rather than jumping immediately. I have not considered these symptoms significant, because they did not occur in cases where the lesion was greater in extent. Because of the heavy band of fibers decussating from the ventral nucleus of the eighth nerve,

particular care was taken in testing for vestibular disturbances. Whether the animal was tested with the optic tract intact or severed, or in a lighted box in a dark room, the responses to vestibular tests were machine like in their accuracy.

In cases where the lesion is high under the cerebellum, the decussating components of the motor paths from the cerebellum are severed. No additional motor symptoms are noted. In one frog, the tractus cerebello-motorius cruciatus was severed after the removal of the cerebellum. No additional disturbances were noted after the second lesion.

It is necessary to conclude, from the preceding analysis of the behavior of frogs with lesions in the mid- and rostral medulla, that severing of 1, the decussating fibers from the ventral and dorsal nucleus of the eighth nerve (bulbar or lateral lemniscus and octavo-motorius cruciatus); 2, the decussating fibers from the cerebellum in the medulla, with or without the removal of the cerebellum, or 3, other components of the internal or external arcuates, has no discernible effect on the behavior of the animal. I have used the word "discernible" because it is possible that laboratory conditions might not reveal some disturbances which would be noticed under more normal environmental conditions.

SUMMARY

The data presented permit us to draw the following conclusions:

Anatomical facts: 1. Marchi preparations of the brains and cords of frogs, in which a slightly lateral lesion of the mid-medulla had been made a month before autopsy, revealed a descending degeneration in the ventral region of the brain stem below the lesion, predominantly on the same side, and extending into the ventral funiculus of the cervical cord.

2. Marchi preparations of the brain and cord of a frog, in which the corpus cerebelli had been removed a month previous to autopsy, revealed a pronounced degeneration in the ventral region of the brain stem and extending down to the thoracic level of the cord.

Physiological observations: 1. The immediate result of extirpation of the corpus cerebelli, and consequent removal of the central terminations of the tractus spinocerebellaris, was a slight change in the ability of the animal to jump from certain heights and depths with as much facility as the normal. No immediate changes in muscle tonus, coördination, or vestibular responses were noted.

2. Unilateral interruption of the tractus spino-cerebellaris produced only the same slight deficiences noted in 1.

3. Twenty-nine out of the thirty-one animals in which uni- or bilateral lesions of the cerebellum were made, exhibited increased extensor tone of the limbs, which terminated, in twenty cases, in strongly convulsive movements or convulsions. The most frequent time of appearance of these

symptoms was during the second post-operative week. The convulsions were weaker and of shorter duration with unilateral lesions.

4. The convulsions produced by cerebellar lesions were not permanently affected by subsequent removal of the cerebral hemispheres.

5. Severing the fibers of the brachium conjunctivum, as they decussate in the caudal midbrain, produced no discernible changes in the behavior of the animal.

6. Severing the decussating components of the tractus cerebello-motorius produced no discernible change in the behavior of the animal.

7. Longitudinal lesions in the central sulcus of the caudal medulla were followed by changes in behavior characterized by four signs of atypical motor coördination: *a*. Turning to either side, although predominantly to the contralateral side if the lesion was lateral. *b*. Inability to withstand sudden and extreme changes in position without losing orientation. *c*. Slow flexion after vigorous extension, most noticeable on the ipsilateral side if the lesion was lateral. *d*. Ataxia and dysmetria, shown in jumping from a box with sides six inches in height, in jumping from objects as low as a foot and a half from the floor, jumping so high that little linear distance is covered, inability to avoid obstacles placed in the way suddenly. These reactions were most obvious in the first week after operation.

8. In fifteen of the animals in which medial longitudinal lesions were made, respiration was not resumed, but there was a striking increase in the force of the heart, accompanied by a constriction of the vessels of the abdominal wall.

9. Medial longitudinal lesions, and lesions slightly lateral to the f.l.m. in the mid-medulla, produced no discernible change in the behavior of the animal.

10. Medial longitudinal lesions in the rostral medulla alone, or combined with cerebellar extirpation, produced no discernible change in the behavior of the animal.

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THE EFFECTS OF ADRENALIN ON THE REFLEX EXCITABILITY OF THE AUTONOMIC NERVOUS SYSTEM[1, 2]

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Received for publication May 8, 1939

That the presence of adrenalin may reduce blood pressure responses to reflex stimulation has been demonstrated by Hoskins and Rowley (1915). Similarly Hsu and Chu (1937–38) showed central inhibitory effects of adrenalin by demonstrating dilatation of the independently perfused spleen and a diminution of the pressor response to stimulation of the floor of the fourth ventricle. This "damping" effect is much reduced after denervation of the carotid sinuses and bilateral vagotomy, but Heymans and associates (1937), as well as Hsu and Chu, showed that a vasodilatation of the independently perfused kidney and extremities also followed adrenalin injection even in the absence of the carotid sinuses.

In view of the fact that observations on the inhibitory action of adrenalin on the autonomic nervous system have been concerned solely with the vascular mechanisms, experiments were carried out to study its action on various branches of the autonomic nervous system. For this purpose the reaction of the pupils, the nictitating membranes, the galvanic (sweat) response and the blood pressure was studied under the influence of adrenalin injection and secretion.

METHOD. Cats anesthetized by urethane and chloralosane or chloralosane alone were arranged as previously described by us (1939) for kymographic recording of blood pressure from one carotid artery, for recording the reactions of both nictitating membranes, for photographic recording of the two pupils, and in some instances for photographing the sweat reactions of the foot pads indicated by a sensitive galvanometer. The recording kymograph was within the photographic field according to the method of Chen, Lim, Wang and Yi (1936).

The preganglionic sympathetic nerve was separated from the vagus

[1] Aided by a grant from The John and Mary R. Markle Foundation.

[2] Preliminary report: Proc. Soc. Biol. Med., **40:** 487, 1939, and Proc. Amer. Physiol. Soc. (Toronto), 1939.

and severed on one side in the neck. Artificial respiration was employed in some experiments. In most instances a moderate intravenous injection of 0.2 to 0.5 cc. of ½ per cent curare (in cats weighing 2 to 2.5 kgm.) was given to reduce bodily movement following stimulation. The femoral vein was cannulated and connected to a burette or to a motor driven continuous perfusion apparatus. The brachial plexus on the side of the

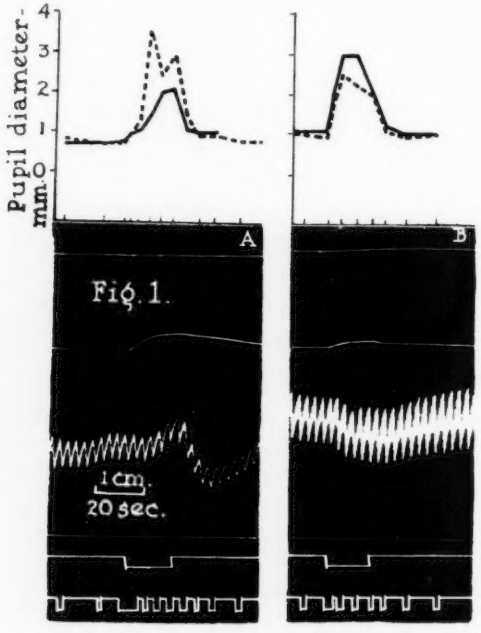

Fig. 1. Influence of adrenalin on the reflex excitability of the autonomic system. The effect of stimulation of the brachial plexus (coil at 5 cm. for 15 sec.) before (fig. 1a) and after (fig. 1b) perfusion with adrenalin 1:200,000 3 cc./min. for 3 min. -----, normal pupil; ——, sympathectomized pupil. Kymograph record from above downward: sympathectomized nictitating membrane (N.M.), normal N.M., blood pressure, signal indicating stimulation of brachial plexus and signal signifying time when photographs of the pupils were taken.

sympathectomy was bared, ligated, and cut, and a shielded electrode applied proximally. An inductorium with two dry cells and a key provided electrical stimulation. A signal marker simultaneously operated by an independent circuit recorded the time of stimulation.

Results. The effects of stimulation of the brachial plexus on pupils, nictitating membranes, and blood pressure under control conditions and after injection of adrenalin in cats acutely sympathectomized on one side

are illustrated in figures 1 and 3.[3] Figure 1 shows that on stimulation of the brachial plexus a slight rise in blood pressure followed by a distinct negative phase occurs. The normally innervated nictitating membrane shows a distinct contraction whereas the denervated nictitating membrane remains unchanged. Both pupils dilate but the dilatation is greater on the normal than on the sympathectomized side. After perfusion of adrenalin 1:200,000 for three minutes we see no pressor response but a slight depressor reaction to stimulation at a somewhat higher blood pressure level. There is no change in the initial[4] contraction of the normal nictitating membrane but the reaction to nerve stimulation is greatly diminished. The denervated nictitating membrane shows only a slight initial contraction due to the injected adrenalin. The pupillary response becomes reversed so that the normally innervated pupil reacts less than the denervated pupil. This reversal is due to an increased reactivity of the denervated pupil (to be considered later) and to a decreased reactivity

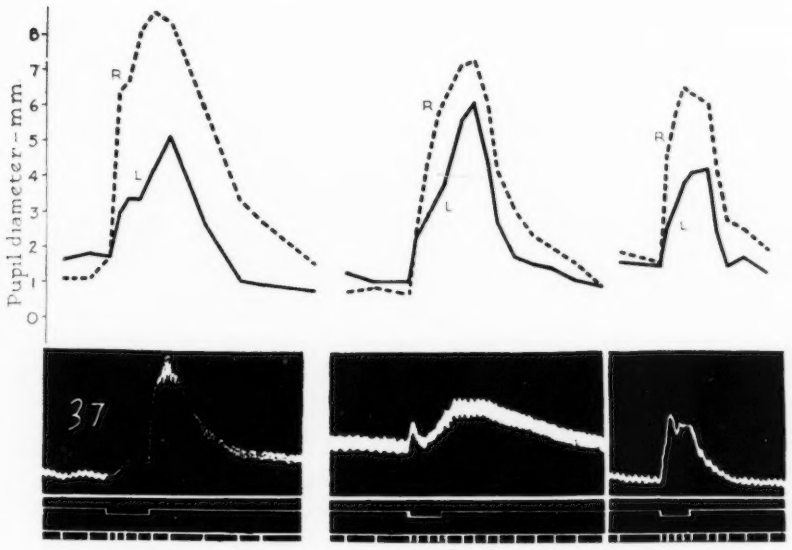

Fig. 2. Stimulation of hypothalamus (coil at 6 cm. for 10 sec.) with the Horsley-Clarke instrument before (left record), during (center), and after perfusion (right record) with adrenalin 1:200,000 (3 cc. perfused in 3.5 min.). Designations as in figure 1.

[3] Observations were made on animals 1 to 4 hours after unilateral sectioning of the cervical sympathetic.

[4] *Initial:* Prior to nerve stimulation.

of the normally innervated pupil suggestive of a decreased sympathetic response. There is no appreciable effect of the adrenalin on the pupillary diameter prior to stimulation. The effect is reversible and within a few minutes after discontinuation of the adrenalin autonomic reactivity typically resembles that preceding perfusion.

Similar effects of adrenalin on reactions to central stimulation within the hypothalamus are shown in figure 2.[5] It is again seen that adrenalin infusion decreases the sympathetic response of the normal pupil and increases the response of the sympathectomized pupil. The effect is reversed on discontinuation of the adrenalin.

The consistency with which these effects may be obtained is indicated by table 1. It is seen that the reaction of the normal pupil to a stimulus

TABLE 1

Effect of adrenalin on the reaction of the pupil to brachial stimulation

NUMBER OF ANIMALS	NORMAL PUPIL			SYMPATHECTOMIZED PUPIL			PUPILLARY DIFFERENCE‡	
	Increased*	Decreased†	No change	Increased	Decreased	No change or doubtful	Increased	Decreased
Effect of perfusion of adrenalin, 0.01 to 0.02 mgm./min.								
10	0	9	1	8	0	2	0	10
Effect of stopping perfusion								
5	4	0	1	0	5	0	5	0
Effect of ligation of adrenals								
8	3	0	5	0	8	0	7	1

* Increased = greater dilatation to brachial stimulation than in the preceding or following control period.

† Decreased = less dilatation to stimulus than in control period.

‡ Pupillary difference = reflex increase in pupillary diameter of the normal pupil minus that of the sympathectomized pupil.

is decreased while that of the sympathectomized pupil is increased by the infusion of adrenalin in the majority of instances, and that in all cases the reaction of the denervated pupil becomes relatively the greater. The opposite effect is seen when adrenalin perfusion is discontinued. Ligation of the adrenals has, in general, the same result as discontinuation of adrenalin although the effect is weaker.

Similar effects were observed in the case of the other autonomic indicators. Nictitating membrane responses were decreased by adrenalin in 7 out of 8 cats, and discontinuation of adrenalin perfusion increased the responses in 5 out of 5 instances where valid comparisons were possible. Blood pressure responses likewise tended to be decreased by the adrenalin.

[5] From an experiment employing the Horsley-Clarke stereotoxic technic, Helen Blake Carlson collaborating.

Galvanic (sweating) responses of the foot pads were generally decreased by adrenalin and increased by ligation of the adrenals.

These effects have been repeatedly observed in the absence of any evidence that adrenalin has increased the tonic activity of the responding mechanism in any way other than by a small increase of blood pressure or pulse pressure. But even the rise of blood pressure is not an absolutely indispensable part of the reaction, for we have occasionally seen the "damping" effect to persist for a period after the blood pressure had fallen to the normal level. The experiments thus show that the sympathetic response is diminished when adrenalin is infused. Such decrease is obtained whether the reflex is induced by faradic stimulation of the central end of the cut brachial plexus, of the sciatic nerve or of the splanchnic nerve. It is frequently observed that when the presence of motor responses in the extremities indicates high somatic excitability, as after metrazol injections with incomplete curarization, or after chlorolosane anesthesia, adrenalin in physiological concentrations reduces this excitability. Similar effects on the knee-jerk have been reported by Schweitzer and Wright (1937).

Figure 3 shows the effects of adrenalin in abolishing or reducing the reflex excitability induced by metrazol. Prior to 3a the blood pressure and nictitating membrane responses had been increased by repeated small injections of metrazol as described by us (1939). In 3b following adrenalin injection the nictitating membrane response is entirely abolished. The blood pressure level is raised by the adrenalin and the blood pressure reaction to a brachial stimulus is reduced.

Since it has been known following the investigations of Bömer (1930) that metrazol convulsions are attended by discharge of adrenalin, it might be expected that this would tend to decrease the reflex responses to stimulation. We found (1939), however, that the facilitating effect of the metrazol on autonomic responses was sufficient to overcome completely the inhibitory or damping effects of the concomitantly secreted adrenalin.

It appears, nevertheless, that adrenalin secretion occurring under various circumstances may have an effect similar to that of perfusion of adrenalin. It was found that after repeated stimulation of the brachial plexus at brief intervals the pupillary difference between the normal and the denervated sides became progressively smaller or was even reversed, and the nictitating membrane response was diminished on the normal side. It was also observed that in the experiments involving urethane narcosis, in which the blood sugar was high (160–180 mgm. per cent) the pupillary difference was small or reversed and the reaction of the normally innervated nictitating membrane was small and galvanic responses were generally lacking. Also on removal of the carotid sinuses which is known to result in release of adrenalin the denervated pupil was invariably larger

and more responsive than the normal one. Furthermore, on ligation of both adrenals the nictitating membrane response and the pupillary difference in response increased. Animals in which brachial stimulation previously had caused equal pupillary dilatation of the normal and sympathectomized eyes typically gave the normal pupillary difference in response after bilateral ligation of the adrenals. In some experiments, galvanic responses from the foot pads appeared for the first time after adrenal ligation.

The experiments indicate as a whole that in adrenalinemia, whether brought about by injection or secretion of adrenalin, the reflex excitability

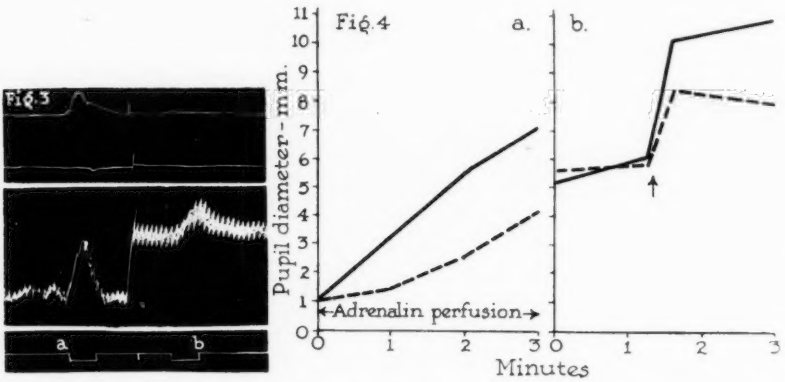

Fig. 3. Influence of adrenalin (0.03 mgm. perfused in 2 min.) after sensitization of the autonomic system with a subconvulsive dosis of metrazol. Prior to figure 3a 0.3 cc. 10 per cent metrazol was injected intravenously. The record of figure 3b was taken during adrenalin infusion. Kymograph record from above downward: normal N.M., sympathectomized N.M., blood pressure, signal signifying brachial stimulation for 15 sec. (coil at 5 cm.).

Fig. 4a. The influence of adrenalin infusion (6 cc. 1:1,000,000/min.) on the normal (-----) and the sympathectomized(——) pupil.

Fig. 4b. The effect of asphyxia clamping of the trachea at ↑ on the normal and sympathectomized pupil.

of the sympathetic nervous system as measured by the reactions of the blood pressure, nictitating membrane, pupils and sweat glands is diminished. Somatic hyperexcitabilty, when present, is also seen to be decreased by adrenalin in physiological concentrations.

Concomitant with these inhibitory or damping effects of adrenalin on reflex sympathetic excitability is the above noted apparent facilitation of reflex inhibition of the parasympathetic tonus in the sympathectomized pupil. Even in the brief period of an acute experiment the sectioning of the cervical sympathetic on one side causes an apparent sensitization of the

denervated pupil to adrenalin. This is generally evident by a persisting negative or paradoxical pupillary difference often occurring in less than one hour after the operation. But even in the absence of an initial pupillary difference, in the presence of adrenalin the sympathectomized pupil may dilate more than the normal one as shown in fig. 4a. A similar effect is seen when stopping of respiration causes increased adrenalin discharge through asphyxia (fig. 4b). The observation that during the inhalation of 6 to 8 per cent oxygen a pupillary dilatation may result which is greater on the denervated than on the normal side seems to warrant a similar interpretation.

Discussion. The experiments reported show that adrenalin decreases the reflex responses of the sympathetic nervous system and thereby leads to a decreased pressor response of the blood pressure, a diminution in the contraction of the normally innervated nictitating membrane, a reduction of the galvanic (sweating) reflex of the foot pads, and a diminution in the response of the normally innervated pupil. The experiments are in line with the observations of Hoskins and Rowley, and Hsu and Chu, cited above, but our experiments show that the damping effect of adrenalin is not restricted to the vasomotor system but affects the sympathetic system as a whole. This conclusion is supported by observations of Malmejac, Donnet and Desanti (1935) who found that the adrenalin secretion is diminished on injection of adrenalin. Moreover, the fact that the removal of the adrenal glands has effects similar to those seen after cessation of perfusion with adrenalin seems to indicate that adrenalin has this damping effect even under physiological conditions. It was further shown that metrazol which stimulates autonomic centers as seen by increased reflex excitability, and adrenalin which increases sympathetic activity by its action on peripheral sympathetically innervated structures, have opposite influences on the reactivity of the sympathetic system.

These observations are entirely consistent with the evidence for "summation of effects" within the autonomic system. Notwithstanding the fact that the concentration-action curves of Wilkie (1928), Rosenblueth (1932), and Acheson and Morrison (1938) were typically obtained in animals deprived of their buffer nerves, the hyperbolic form of the curve would indicate decreasing effects from successive increments of adrenalin or sympathin. The decrement is unquestionably greater when buffer damping mechanisms are present.[6]

In contrast to this "damping" of sympathetic excitability by adrenalin we have also noted the increased dilatation of the sympathectomized pupil

[6] A factor reducing sympathetic responses by other than reflexes from the buffer nerves has recently been suggested by the findings of Marazzi (1939) who shows inhibitory effects of adrenalin on the transmission of impulses through sympathetic ganglia.

in response to a stimulus. Bain, Irving and McSwiney (1935) and Ury and Gellhorn (1939) have demonstrated the large rôle played by reflex inhibition of parasympathetic tonus in the reflex pupillary dilatation following afferent stimulation. This reflex inhibition we have here shown to be increased by the presence of adrenalin. This augmentation may involve one or more of the following mechanisms:

1. Tonic parasympathetic impulses to the denervated pupil may be decreased. If this is the case we have the difficulty of explaining how this may occur homolaterally.

2. The effect may be due to a sensitization in the denervated structures similar in kind but less in degree than that which has been noted in the literature after long intervals (cf. Cannon and Rosenblueth, 1937). Such a sensitization is indicated by the greater effect of adrenalin injection on the denervated pupil (fig. 4). There is, however, the difficulty of explaining how in this case the increased reflex reactions of the sympathectomized pupil can occur in phase with those of the normal pupil. They take place with such rapidity (within 2 to 4 sec.) as to make it improbable that the increase is due to a momentarily increased reflex adrenalin secretion by the organism or even due to sympathin secreted by the nearest structures with an intact sympathetic supply.

3. It may be due to adrenalin interfering with the action of acetylcholin in the pupillo-constrictors and thereby rendering a given degree of inhibition more effective. Such an antagonism between adrenalin and acetylcholine possibly contributes to the decreased sweat gland (galvanic) responses in this experiment. Similar effects of adrenalin on sweating have been reported (Darrow, 1937) and inhibitory effects of adrenalin on cholinergic mechanisms such as the gut are well established.

Since it is shown that the effect of adrenalin infusion is similar to that of adrenalin secretion, the experiments warrant a physiologic interpretation. Cannon, following Claude Bernard, has established the principle of homeostasis according to which regulations should occur which reestablish the constancy of the internal environment. The experiments presented in this paper support this idea by showing, especially in the animal with intact buffer nerves, that the secretion of adrenalin into the circulation is itself an effective damping mechanism for reducing the reactions induced by sympathetic excitation. The secretion of adrenalin following a strong excitation of the sympathetic centers seems to be a mechanism helping to restore the system to a normal excitability.

CONCLUSIONS

The injection of adrenalin or the liberation of adrenalin from the adrenal glands leads to a diminished reflex excitability of the sympathetic nervous system. The blood pressure response to stimuli applied to afferent nerves

is diminished or disappears completely, the contraction of the normally innervated nictitating membrane is lessened and the difference between reflex pupillary dilatation on the normal and sympathectomized sides following afferent stimuli is diminished or reversed. The galvanic (sweating) response may likewise be decreased. In the case of the parasympathetically innervated sympathectomized pupil the inhibitory effects of reflex stimulation are increased. The experiments thus indicate an inhibitory action of adrenalin on mechanisms controlled by both branches of the autonomic system. The effect on reflex excitability is opposite to that of metrazol and the two pharmacologic agents may counteract one another in this respect. The significance of these observations is discussed from the point of view of "homeostasis" and "emergency function."

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THE PROPERTIES OF MAMMALIAN B FIBERS

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Received for publication May 13, 1939

The elevation in the electroneurogram of mammalian autonomic nerves that is propagated at velocities from 15 to 3 m.p.s. was described by Bishop and Heinbecker (1930) and called by them the B_2 spike. The group of fibers producing this elevation forms the object of the present investigation, the purpose of which is twofold: first, to enlarge the basis upon which it was established by Bishop and Heinbecker that these fibers constitute a separate group with distinctive properties; and secondly, to study these properties more intensively from the point of view of their possible relationship to the general pattern exhibited by the action potential of A fibers (Gasser and Grundfest, 1936) and of the C fibers (Grundfest and Gasser, 1938). The terminology employed is that suggested by Erlanger (Erlanger and Gasser, 1937, Chap. II), in which the subscript of B_2 is dropped. These fibers of the autonomic system are thus designated as "B" in the present paper.

Differentiation of the B group from other groups of nerve fibers was made by Bishop and Heinbecker on the basis of distinctive ranges of velocity and threshold, rising time of the unconducted spike and duration of the absolutely refractory period. The evidence of Bishop and Heinbecker has been corroborated, and it has been possible to add a number of other properties in regard to which the B fibers differ from those of the faster A and the slower C group. These properties are the spike duration, the excitability cycle, the after-potential sequence and its modifications under conditions of activity and environmental change. Certain numerical discrepancies that are found to exist between the values given by Bishop and Heinbecker and those reported here are probably attributable to two factors. In the first place, since the publication of their earlier work, improvements in oscillographic methods have obviated the necessity for frequent stimulation of the nerve in order to obtain distinct photographic records. The nerves used in the present experiments were, therefore, less likely to be subject to the conditioning effects of previous activity. Secondly, the nerves were kept under conditions of temperature, pH, and ionic environment which are now standard in this laboratory (Lehmann, 1937a, b), but which did not obtain in the older work. For these

reasons, the discrepancies mentioned may be considered as being minor in character.

The majority of the data were obtained on the B fibers of the hypogastric nerve of the cat; they were supplemented by observations made on the cervical sympathetic nerves of the cat and the rabbit. With the exception of one type of experiment, all the measurements were made upon excised nerves. These were placed on Ag-AgCl electrodes in a moist chamber kept at 37 to 38°C., into which was bubbled a stream of 5 per cent CO_2 in O_2. While in the chamber, the nerve, without removal from the electrodes, could be dipped into a bath of Krebs' solution. The nerve was thus maintained at pH 7.4 and with a standard ionic balance. In most of the measurements a fast direct-current amplifier designed by Doctor Toennies and regularly employed in the laboratory was used. Stimulation was effected by one or more thyratron-controlled condenser discharges delivered to the primary of a special transformer. Low coupling capacity between the primary and the secondary that applied the stimulus to the nerve reduced the shock escape considerably.

In the hypogastric nerve the B spike is relatively small, amounting after 1 cm. conduction to only about 0.5 mv. in the best preparations, while the C spike is usually 4 to 6 times this amplitude. Nevertheless, the hypogastric nerve offers the most favorable source for the study of B fibers, because usually there is still less and often almost no spike potential from more rapidly conducting fibers (fig. 1). In contrast to the small, but comparatively pure B potential of the hypogastric nerve, the B spike of the cervical sympathetic nerve of the cat has a relatively high amplitude, but it is always preceded by a faster delta spike (fig. 4) usually of about equal magnitude. There is in both nerves an overlapping of thresholds between delta and B at the one end, and often also between B and C at the other.

The spike duration. The difficulties that are involved in the recording of single-fiber responses from threshold B fibers need not be reëmphasized here. In addition to the small amplitude of potential that may be expected from single B fibers, the presence of responses from the larger A or the delta fibers usually further complicates the picture. Figure 2 shows a threshold response from the cervical sympathetic nerve of the rabbit, which was used because this nerve is most often free of faster components. The spike duration in this nerve, as well as in various preparations from the cat, is of the order of 1.2 msec., thus giving a wave length of about 1.5 cm. for the fastest B fibers. Again, like in the A and the C fibers, the spike duration as measured is correlated closely to the duration of the absolutely refractory period, which in a number of experiments varied from 1.1 to 1.5 msec. (fig. 13).

The after-potential. The action potential in single responses of B fibers

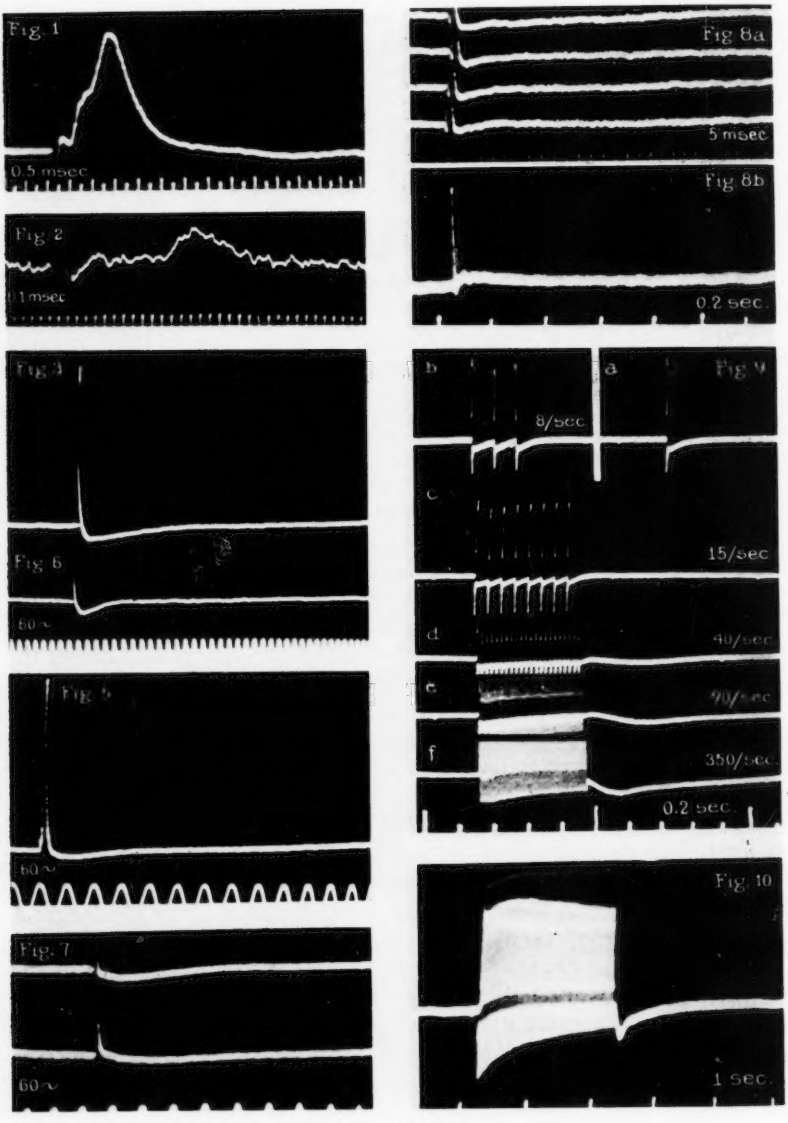


Fig. 1
0.5 msec
Fig. 2
1 msec
Fig. 3
Fig. 6
60~
Fig. 5
60~
Fig. 7
60~
Fig 8a
5 msec
Fig 8b
0.2 sec
b
a
Fig 9
8/sec
c
15/sec
d
40/sec
e
90/sec
f
350/sec
0.2 sec
Fig. 10
1 sec.

Figs. 1-3, 5-10

(fig. 3) is distinctively characterized by the absence of any visible negative after-potential. In a single action the spike negativity is immediately followed by a deep positive after-potential which reaches its maximum within 20 to 30 msec. and subsides in the hypogastric and cervical sympathetic nerves of the cat to reach the resting level of potential 100 to 300 msec. after the initiation of the spike. At its maximum the amplitude of the positive after-potential attains 30 to 50 μv., or about 10 to 15 per cent of the recorded height of the spike. However, when in these slowly conducting fibers correction is made for the dispersion of the spike potential, the positivity becomes 1.5 to 4 per cent of the unconducted B spike. Even at the latter range of values, the positive after-potential of the B fibers is proportionately about tenfold that found in the A group. Figure 4c shows the characteristic increase of the positive after-potential when only a small part of the B spike is added to a preceding delta action of a cervical sympathetic nerve of the cat.

The amplitude of the positive after-potential is equally large in the B fibers of the cervical sympathetic nerve of the rabbit (fig. 5), and the transition from spike negativity to after-positivity likewise takes place without any sign of an intervening negative after-potential. However,

Fig. 1. B spike in the hypogastric nerve of the cat. The B response, which has a velocity of propagation of 12 m.p.s. and less, rises out of a smaller preceding elevation produced by fibers conducting at about 20 m.p.s. Potential of spike, 0.2 mv. Conduction distance, 15 mm. In this and in the succeeding records the figures above the time scale refer to the smallest divisions.

Fig. 2. Threshold B spike at high amplification. Cervical sympathetic nerve of the rabbit. The potential is about 25 μv. Conduction distance, 15 mm. Velocity of conduction, 12 m.p.s.

Fig. 3. The B action potential at pH 7.4. Hypogastric nerve of the cat.

Fig. 5. B action potential of the cervical sympathetic nerve of the rabbit. Conduction distance, 16 mm.

Fig. 6. B action potential of the hypogastric nerve at an alkaline reaction. The pH is approximately 8.0, obtained by substituting pure O_2 for the usual gas mixture of 5 per cent CO_2, 95 per cent O_2. Figure 3 shows the action potential of the same nerve at pH 7.4.

Fig. 7. Effect of an acid reaction on the action potential of the B fibers of the hypogastric nerve. Upper record at pH 7.4; lower record after 1 min. at pH about 6.8.

Fig. 8. Course of the development of a negative after-potential in a veratrinized hypogastric nerve. *a*, Upper record before; others at 1 min., 3½ min. and 6¼ min. after wiping the nerve with Krebs' solution containing 1:10,000,000 of veratrine. *b*, Contralateral nerve 5 min. after applying veratrine, 1:2,000,000. Note that the time base is much slower.

Fig. 9. Modification of action potential of B fibers by repetitive activity. Hypogastric nerve of the cat. *a*, Single response. *b* to *f*, Repetitive responses at the frequencies indicated above each record.

Fig. 10. Course of the after-potential in B fibers of the hypogastric nerve after prolonged activity. Same preparation as in figure 9.

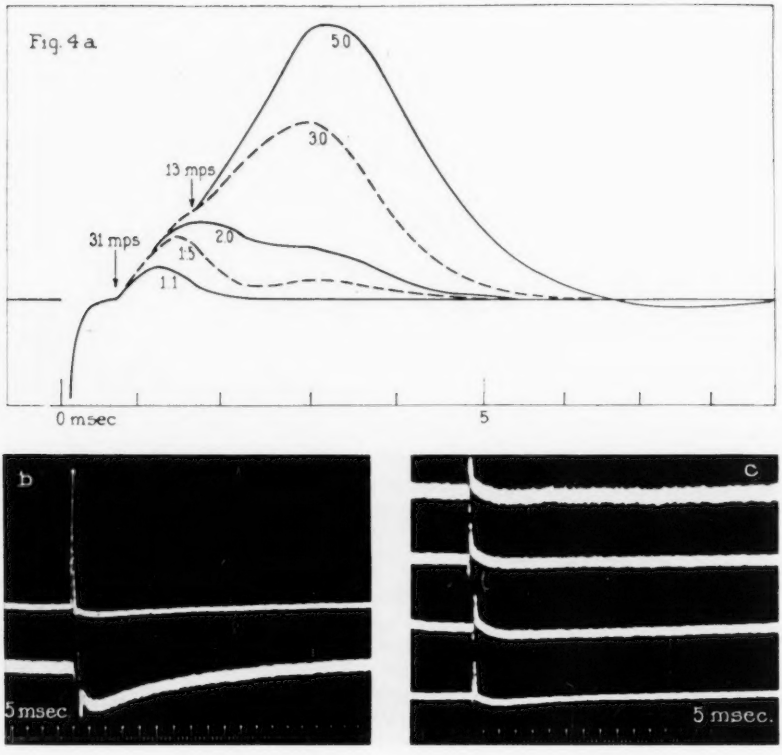
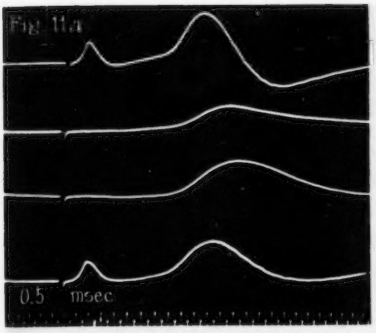
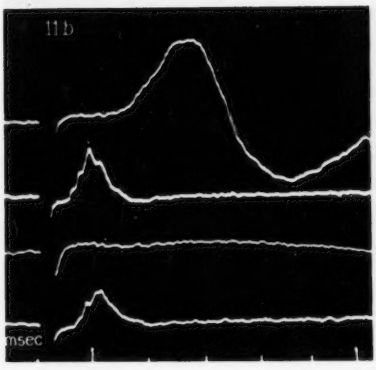


Fig. 4. The action potential of the cervical sympathetic nerve of the cat at different strengths of stimulation. *a*, Superimposed tracings of the spike produced at 5 stimulus strengths. The number inside each tracing gives the relative strength of stimulation, threshold being 1.0. At strength 1.5 a slight B response is observed. *b*, Complete action potential at stimulus 5.0. Upper record at low amplification; lower record at 7.5 × that amplification. *c*, After-potentials of the responses at strengths 1.1, 1.5, 2.0, and 3.0. The amplification for each record was adjusted so that the heights of the spikes remained constant within 2 per cent. In the upper record a negative after-potential and a short small positive after-potential are observable. At strength 1.5 a prolongation of the positive after-potential is visible; this can be attributed to the very small increment of B fibers shown to occur at this strength of stimulus (fig. 4a). In the lowest record, taken at strength 3.0, the negative after-potential of the delta fibers has been outweighed by the positivity of the B fibers. Conduction distance, 22 mm. The delta component of this nerve was exceptionally small.

the duration of the positive after-potential of rabbit B fibers is much less than it is in nerves of the cat, and the resting level is reached after 60 to 100 msec.





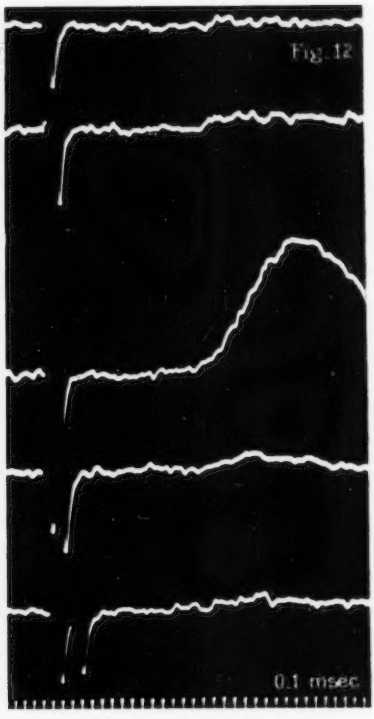


Fig. 11. Differential sensitivity of A, B, and C fibers to asphyxiation. *a*, Hypogastric nerve of the cat. Shock maximal for C. In the upper record is the combined response of B and C fibers before asphyxiation was begun. In the second line the B spike is completely absent, while a considerable but slowed and dispersed C spike still remains. The next two lines show the recovery, first of the C spike at 1 min., and then of both the B and C spikes at 2 min. after re-introducing O_2. *b*, Cervical sympathetic and depressor nerves of the rabbit were placed on common leading electrodes, but were stimulated independently. Upper record, B spike of the cervical sympathetic nerve; below it the A spike of the depressor nerve before beginning asphyxiation. The third line shows the nearly complete absence, due to asphyxiation, of the B spike on stimulation of the cervical sympathetic nerve, while the response produced by stimulating the depressor nerve is still large (fourth line).

Fig. 12. Latent addition in B fibers. Cervical sympathetic nerve of the rabbit. Reading downward: Conditioning response in isolation; test response in isolation; both applied nearly simultaneously; both at an interval of 0.1 msec.; at an interval of 0.2 msec.

In the B fibers of the cat a shortening of the positive after-potential is a primary step in the modification of the action potential under changes of specific environmental conditions.

Effect of changing the pH. A shortening of the positive after-potential is observed when the pH is increased, as the B fibers are particularly sensitive to this modification of their environment. Within a minute after the gas bubbling into the moist chamber is modified by removal of the CO_2, thus increasing the pH, the after-potential of cat B fibers begins to shorten and finally it lasts only 60 to 80 msec. (fig. 6). The change is reversed with nearly equal rapidity when the standard gas mixture is restored. On the other hand, a lowering of the pH by increasing the percentage of CO_2 in the gas mixture has the effect shown in figure 7. The transition of the action potential into after-positivity is somewhat slowed by an overt manifestation of a transitional negative after-potential; the amplitude of the positive after-potential is decreased, but the duration remains unaffected.

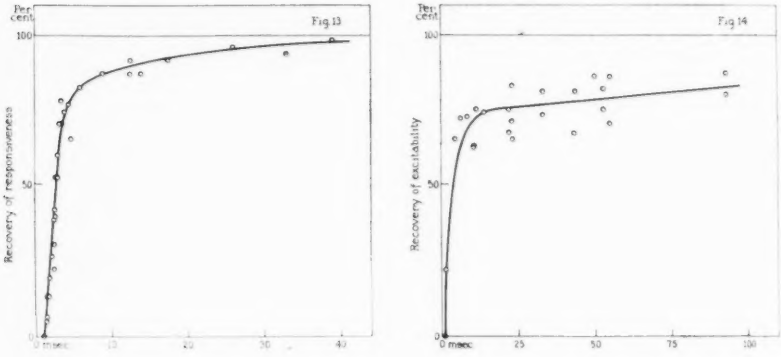


Fig. 13. Recovery of responsiveness in the hypogastric nerve of the cat
Fig. 14. Recovery of threshold in an excised hypogastric nerve

Veratrine. The existence in B fibers of a mechanism capable of producing large amounts of negative after-potential is easily shown by veratrine. Within 6 minutes after painting the nerve with 1:10,000,000 veratrine, the action potential goes through the sequence of changes shown in figure 8a. The positive after-potential at first is shortened from behind and then completely obliterated as after-negativity wells up to persist for more than 1 second in the fully veratrinized nerve.

Repetitive activity. During repetitive activity the successive responses individually contribute less positive after-potential (fig. 9b) than does the first action (fig. 9a). The decrease in newly formed positivity is first marked by a shorter duration of the positive after-potential terminating the last response. A decrease in the depth of positivity also takes

place and becomes more marked as the train of responses is prolonged, and the activity may end with only a small positive after-potential (fig. 9c) or even on a negative level (fig. 9d). As the amount of terminating positivity is decreased, there is a development of later positivity (fig. 9d, e) which first arises about 200 to 300 msec. after the end of the train of spikes. This positive after-potential becomes more pronounced, beginning earlier and ending later (fig. 9f), when the extent of repetitive activity is enlarged. Following tetanic activity of several seconds' duration, the sequence of after-potentials is that shown in figure 10. At the end of the spike production there are developed a sharp positive notch lasting about 0.2 to 0.3 second and then a period of positivity during which the return toward the resting potential is more gradual. Because the potentials dealt with are small in magnitude, it is difficult to follow the course of this late positive after-potential for more than 1 or 2 seconds.

Effect of asphyxia. B fibers are more sensitive to a deprivation of oxygen than are the fibers of the other groups. Figure 11a shows the differential disappearance of the B spikes in a hypogastric nerve when a considerable portion of the C spike still remains. From the same figure it is also seen that after complete asphyxiation of both spikes, the C fibers recover earlier than do the B fibers.

In order to investigate the relative rates of asphyxiation of B fibers and of slow A fibers, use was made of a preparation consisting of the excised cervical sympathetic and depressor nerves of the rabbit. The two nerves were dissected free of one another for about half their length, so that a separate pair of stimulating electrodes could be applied to each. A single pair of leading electrodes was common to both nerves. Figure 11b shows that the B spike of the cervical sympathetic nerve is practically completely asphyxiated at a time when the response of the A fibers of the depressor nerve is only slightly reduced.

As in the case of fibers of the other groups, the sensitivity of B fibers to asphyxiation is enhanced after recovery from a previous asphyxiation.

Excitability of B fibers. Latent addition. While in C fibers the period of latent addition is about 2 to 2.5 msec. (unpublished) or 10 times as long as that of A fibers, the value for B fibers is identical with that of A fibers. Figure 12 shows the records from one experiment on a cervical sympathetic nerve of the rabbit.

Recovery of responsiveness in B fibers follows a course shown by the curve of figure 13. After a period of absolute refractoriness, which lasts for 1.1 to 1.5 msec. (10 experiments), the height of the response to thrice maximal stimuli increases rapidly, reaching 70 to 80 per cent of the height of the unconditioned response. In this respect, the recovery of responsiveness in B fibers follows a course which is similar to that taken by A and C fibers. Complete recovery of the ability to respond does not, however,

take place until after 40 to 50 msec. have elapsed, a course which may be correlated in B fibers with the lack of negative after-potential. The effect of this slow recovery of responsiveness is shown in the records of figure 9, where even at relatively low frequencies of repetitive activity the height of the second response is considerably smaller than is that of the first.

Recovery of excitability takes a course (fig. 14) different from that of recovery of responsiveness, and one which may be predicted on the basis of the after-potential sequence. During the first 5 msec. after a conditioning action, the excitability rises steeply and attains 60 to 80 per cent of normal. The return to normal excitability then becomes very gradual and complete recovery is not attained until 120 msec. or longer have elapsed. In B fibers, excitability thus remains subnormal during the period of the positive after-potential, as it does in nerves of the other groups.

As has been shown by a series of reports from this laboratory, the conditions under which it is now possible to maintain excised mammalian nerves approximate closely those obtaining in the body, so that the data here presented from a study of excised material may be carried over to nerves *in situ*. Furthermore, because of the smallness of the B spike, the measurement *in situ* of the recovery of excitability is accompanied by technical difficulties. In the most successful of the experiments on the hypogastric nerve *in situ* the recovery of excitability was followed for 40 msec. after the conditioning action. At that interval, recovery was only 40 to 50 per cent complete. The experimental procedures used to insure conditions within the normal physiological range have been described by Grundfest and Gasser (1938).

Although the B fibers differ from A fibers in all the physiological characteristics described except that of latent addition, histologically they form (Bishop and Heinbecker, 1930) the lower part of the continuum of myelinated fibers. In our best preparation of the rabbit cervical sympathetic nerve the myelinated fibers had diameters below 3 μ, except for several larger fibers (7 to 6 μ), the presence of which was foretold in the action potential, where a very small elevation conducted at 40 m.p.s. preceded the large B spike which had a maximal velocity of 14 m.p.s.

That B fibers occur in the preganglionic autonomic nerves is obvious from the original description of them in the cervical sympathetic trunks. Myelinated fibers have also been described in the postganglionic cervical sympathetic system, and the short ciliary nerve (Christensen, 1936) contains many fine myelinated fibers. No potential studies have been made on ciliary nerves. Potentials have, however, been recorded from leads on the ciliary ganglion (Whitteridge, 1937). The potentials which Whitteridge describes are similar to those found in the action potential of B fibers.

DISCUSSION. The data which have been presented in the earlier sections show that the B fibers of mammalian preganglionic nerves differ qualitatively from the A and the C fibers with respect to the form of the action potential and the various modifications that the action potential may undergo in response to experimental modifications of the environment. The distinction drawn by Bishop and Heinbecker between the B fibers and the fibers of the other groups, a differentiation which was made chiefly on the basis of a distinctive range of thresholds, velocities, and refractory periods, is accordingly confirmed by other types of evidence.

The present data permit furthermore an analysis of the extent to which the B fibers in their behavior resemble the responses characteristic of the A and the C fibers. The analysis shows that the elements of which the action potential of B fibers is composed are the same as those which are involved in the action potentials of the fibers of the other groups. According to the generalized schema developed by Gasser (1937) and applied also to mammalian C fibers (Grundfest and Gasser, 1938), the spike is succeeded by concurrent negative and positive after-potentials, the summation of which determines the chief characteristics of the action potential sequence of the single response. Following and dependent upon the negative after-potential is a late positivity, which is small in the single action but increases when the negative after-potential is augmented.

In the following discussion the potential sequence of the action potential of B fibers, and the changes developed in this sequence during and after repetitive activity (figs. 9 and 10), are interpreted on the basis of this schema. As veratrinization and other procedures show, a negative after-potential is present in B fibers, but in the normal single action (9 a) the negativity is outweighed by the first positive after-potential, P_1. Repetitive activity is one of the procedures that bring out the negative after-potential. The manner of the appearance of the potential depends upon the length and frequency of the tetanus. Mild tetanization causes the negative after-potential to be augmented faster than is P_1. If the tetanization is very mild, the only visible result is a progressive apparent curtailment in P_1 and a decrease in its size (fig. 9 b, c). After a somewhat greater tetanization overt negativity appears following the curtailed P_1 at the end of the train of responses (9 c, d). Development of the negative after-potential brings with it in its wake the second positive potential, P_2 (9 d, e). As the frequency of the tetanization is increased, the augmentation of P_1 begins to gain on that of the negative after-potential and P_1 again becomes visible at the end of the tetanus (9 f). At this stage the negative after-potential becomes lost to view and the only indication of its existence is the developed P_2 with which P_1 is merged. Prolonged activity (fig. 10) causes P_1 to appear as a sharp notch in the potential picture. The

size of the potential is augmented, but the duration is still that of the potential seen after a single action, about 0.3 sec. P_2 increases both in size and duration as the severity of the tetanus is increased.

SUMMARY

The properties of the fibers contributing to the B elevation of the action potential of preganglionic autonomic nerves are quantitatively so different from those of other fibers as to justify the designation of the B group as an entity qualitatively distinct from the A and the C groups.

The duration of the spike (1.2 msec.), the size and duration of the positive after-potential (1.5 to 4 per cent of the spike height, duration 100 to 300 msec.), the absence of negative after-potential in single responses, and the form of the recovery curve after excitation are definitive for the group. In all respects B fibers are sharply set off from C fibers. But they have some points of resemblance to A fibers. The period of latent addition (0.2 msec.) is the same for the two. With respect to the refractory period and the sensitivity to asphyxia the B fibers come into line with the small A fibers. The B fibers also resemble A fibers in their sensitivity to pH changes.

The basic pattern of the after-potential changes exhibited by B fibers is similar to that found in A and C fibers. Although a negative after-potential is lacking in the normal single action, it appears during and after periods of repetitive activity. Following a prolonged tetanus, the after-potentials have the same form for all fibers, only the characteristic configuration appears on different time scales, depending upon the group of fibers in question.

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LIBERATION OF ACETYLCHOLINE AND ADRENALINE BY STIMULATING ISOLATED NERVES[1]

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Received for publication May 15, 1939

In recent publications from this laboratory (Lissák, 1938, 1939) it was shown, as has been reported by others, that various cholinergic nerves and ganglia contain acetylcholine in different amounts. Furthermore, evidence has been presented by Lissák (1938, 1939) and by Cannon and Lissák (1939) that adrenaline can be extracted from adrenergic neurones, mixed nerve trunks which contain postganglionic sympathetic fibers and from organs like the heart and liver, which contain adrenergic nerve fibers and terminals. Numerous investigators (Calabro, 1933; Binet and Minz, 1934, 1936; Bergami, 1936, 1938; Bettencourt and Paes, 1936; Babsky and Kisliuk, 1938) have reported that *in vitro* stimulation of various nerves liberates acetylcholine and probably an adrenaline-like substance. By stimulating *in vitro* the vagus nerve of the dog Binet and Minz (1934, 1936) were able to detect a substance which sensitizes the leech muscle to acetylcholine. Further investigations by Minz (1938) indicated that this substance may be vitamin B_1.

The experiments mentioned above on the liberation of chemical agents by stimulation of nerve elements do not allow a separation of the fibers which release acetylcholine from those which produce an adrenaline-like agent—i.e., the data do not establish whether cholinergic nerve fibers liberate only acetylcholine when stimulated and whether adrenergic fibers liberate exclusively adrenaline. The observations on the release of adrenaline are particularly incomplete. The present study was planned to furnish more information on this problem.

METHOD. Cats anesthetized with dial (Ciba, 0.7 cc. kgm. intraperitoneally) and frogs were used. The nerves to be examined were isolated and removed, washed and kept in Ringer solution containing physostigmine (1:100,000), or in simple Ringer solution. When stimulated they were submerged in 1 cc. Ringer solution and attached to silver electrodes. The region of the nerve where the electrodes were applied was kept moist. Care was taken that no fluid could run down the nerve from the inter-

[1] A preliminary publication appeared in the Proceedings of the American Physiological Society, 1939.

electrodal stretch to the Ringer's in which the nerve was submerged, thus excluding the objections of Gaddum, Khayyal and Rydin (1937). The stimuli were supplied by a Harvard inductorium, with one dry cell in the primary circuit and a secondary-coil distance of 5 cm. After stimulation the solutions were tested on the isolated frog heart. For the presence of a positively acting adrenaline-like substance the solutions were tested on the hypodynamic frog heart. Such tests were made with or without previous dialysis of the solutions. The presence of acetylcholine was determined

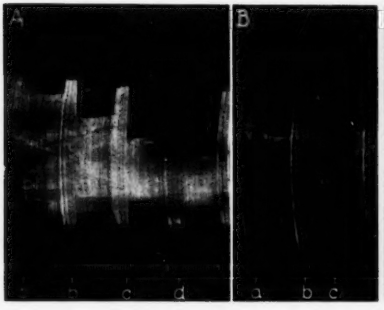

Fig. 1

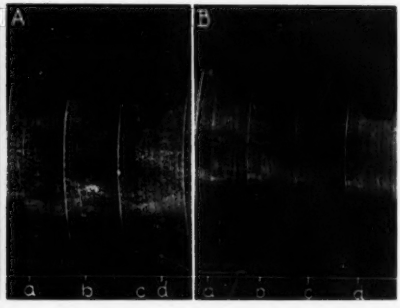

Fig. 2

Fig. 1A. Normal frog heart. In this and succeeding records the time signal marks 5-second intervals. Nerves were stimulated in 1 cc. physostigmine-Ringer. At *a*, acetylcholine (a.ch.) 0.001 γ; at *b*, mid-part of frog's sciatic submerged, one end stimulated 10 minutes; at *c*, two cut ends of frog's sciatic submerged, mid-part stimulated 10 minutes; at *d*, a.ch. 0.01 γ.

B. At *a*, mid-part of frog's sciatic submerged, one end stimulated 10 minutes; at *b*, same as at *a*; at *c*, atropine 2 drops from a solution 1:10,000.

Fig. 2A. Normal frog heart. At *a*, mid-part of cat's vagus submerged, one end stimulated 10 minutes; at *b*, two cut ends of cat's vagus submerged, mid-part stimulated 10 minutes; at *c*, superior cervical ganglion of cat preganglionically stimulated 10 minutes; at *d*, atropine 2 drops from a solution 1:10,000.

B. At *a*, phrenic of cat, mid-part submerged, one end stimulated 10 minutes; at *b*, a.ch. 0.005 γ; at *c*, a.ch. 0.01 γ; at *d*, phrenic of cat, two cut ends submerged, mid-part stimulated 10 minutes.

without dialysis on normal frog hearts—i.e., before they became hypodynamic. If the nerve trunks of the cats were stimulated in mammalian Ringer, the solutions were diluted (1:1.4) before testing with distilled water to make frog Ringer. For quantitative comparison, titrations with acetylcholine (acetylcholine chloride, Merck) and with adrenaline (adrenalin, Parke, Davis) were made on the same hearts.

Results. A. *In vitro stimulation of cholinergic nerves and sympathetic ganglia.* The isolated cholinergic nerves used were frogs' sciatics and cats' sciatics, cervical vagus and sympathetic, phrenic, and the superior

cervical and nodosum ganglia. The nerves were placed in 1 cc. physostigmine-Ringer solution so that the two cut ends were submerged and the mid-portion was stimulated outside the solution. After 10 minutes of stimulation the solutions were tested on the Straub heart. Figures 1, 2 and 3 show the acetylcholine-like negative action of the solutions and of acetylcholine used for comparison.

When, instead of dipping the cut ends of the nerves, the mid-portion was submerged in the solution and one end stimulated for the same period of time (10 min.), about half as much acetylcholine-like substance was liberated (figs. 1, 2, 3). There was no difference in the amount of acetyl-

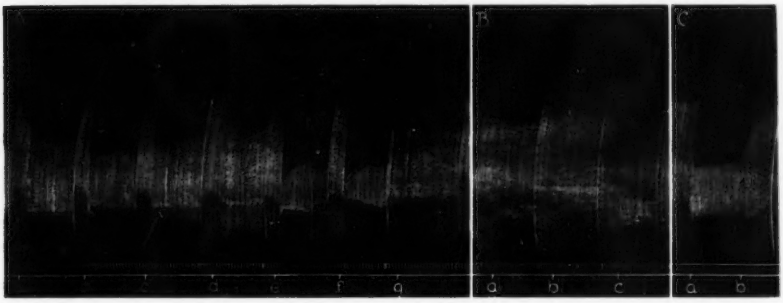

Fig. 3A. Normal frog heart. At *a*, sciatic of cat, mid-part submerged, one end stimulated 10 minutes; at *b*, a.ch. 0.005 γ; at *c*, sciatic of cat, two cut ends submerged, in physostigmine-Ringer without stimulation 40 minutes; at *d*, same as at *c*, but 20 minutes; at *e*, sciatic of cat, two cut ends submerged, mid-part stimulated 10 minutes; at *f*, a.ch. 0.01 γ; at *g*, sciatic of cat, mid-part submerged in Ringer without physostigmine, one end stimulated 10 minutes.

B. At *a*, cat sciatic, mid-part submerged, one end stimulated 10 minutes; at *b*, cat sciatic transected one week previously, mid-part submerged, one end stimulated 10 minutes; at *c*, a.ch. 0.005γ.

C. At *a*, sciatic of cat, mid-part submerged, one end stimulated 20 minutes; at *b*, atropine 2 drops from a solution 1:10,000.

choline liberated whether the peripheral or the central end of the nerves was stimulated.

If the cut ends were immersed in Ringer's for a longer time (20 to 40 min.), even without any stimulation some acetylcholine-like substance appeared in the solution (fig. 3Ac, d). On the other hand, no acetylcholine was detected when the mid-part was submerged for the same time.

Some nerves were tested for release of acetylcholine upon stimulation at different time intervals after section and consequent degeneration. In cat's sciatics the results were the following. One day after the operation no difference was detectable in the amounts of acetylcholine-like substance liberated by stimulation of the normal and the cut nerve. After two or

three days' degeneration only about half as much substance was liberated as from the control normal nerve. After one week of degeneration the nerve does not liberate any acetylcholine-like substance (fig. 3Ba, b). Similarly the superior cervical ganglion does not liberate any acetylcholine when stimulated one week after preganglionic denervation.

Control experiments showed that the substance liberated from the cholinergic nerves and ganglia stimulated *in vitro* is a choline ester, probably acetylcholine. Its negative action on the frog's heart was abolished by atropine (1:100,000) (figs. 1, 2, 3). The liberation of the substance did not take place without the presence of physostigmine (fig. 3Ag). The substance contracted the eserinized leech muscle and rectus abdominis of the frog.

In the case of mammalian nerves there was no significant difference in the amount of acetylcholine liberated whether the nerve was stimulated in frog's Ringer, or in mammalian Ringer (before diluting for test).

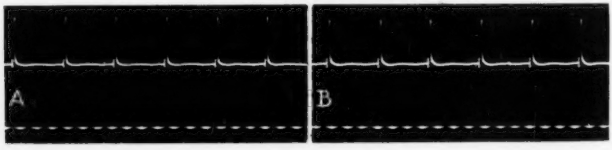

Fig. 4. Electric responses of cat sciatic nerves. Monophasic recording; cathode-ray oscillograph. Time signal: 10 msec. A. Nerve freshly excised. B. Opposite nerve after an experiment during which stimulation was applied for 30 minutes.

That the stimuli did not result in any damage of the nerve even if applied for longer periods than those reported above was shown by the normal electric responses recorded from some nerves at the end of the experiment. Figure 4A shows the action potentials of a sciatic of the cat recorded at the beginning of one of the experiments; and figure 4B illustrates the responses of the opposite nerve after it had been stimulated *in vitro* for 30 minutes.

Titrations were made with acetylcholine on the frog heart before and after testing the solutions in which cholinergic nerves (vagus and sciatic of the cat, sciatic of the frog) had been stimulated. Contrary to the observations of Binet and Minz (1934, 1936) on leech muscle, stimulating cholinergic nerves did not cause the appearance of a substance which sensitizes the frog heart to acetylcholine. Treatment of the frog heart with different concentrations of vitamin B_1 or B_2 (kindly supplied by Dr. Y. Subbarow) and with extracts of yeast, produced no sensitization to acetylcholine. Such an experiment is illustrated in figure 5.

B. *In vitro stimulation of adrenergic nerves.* In order to have pure postganglionic sympathetic fibers it was necessary to perform preliminary sterile operations on the animals used. It was already mentioned in the

previous section that the preganglionically denervated superior cervical ganglion of the cat does not yield any acetylcholine when stimulated

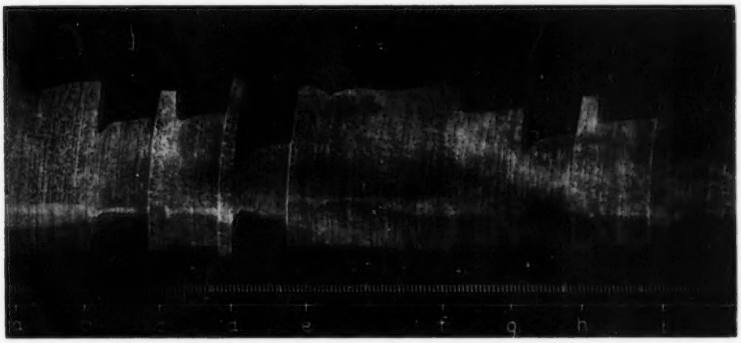

Fig. 5. Normal frog heart. At *a*, a.ch. 0.001 γ; at *b*, a.ch. 0.005 γ; at *c*, a.ch. 0.001 γ; at *d*, a.ch. 0.01 γ; at *e*, vitamin B_1 in conc. 1:10,000; at *f*, a.ch. 0.001 γ; at *g*, a.ch. 0.005 γ; at *h*, a.ch. 0.001 γ; at *i*, a.ch. 0.01 γ; after each record (*f*, *g*, *j* and *i*) the heart was washed with Ringer's solution containing vitamin B_1 in conc. 1:10,000.

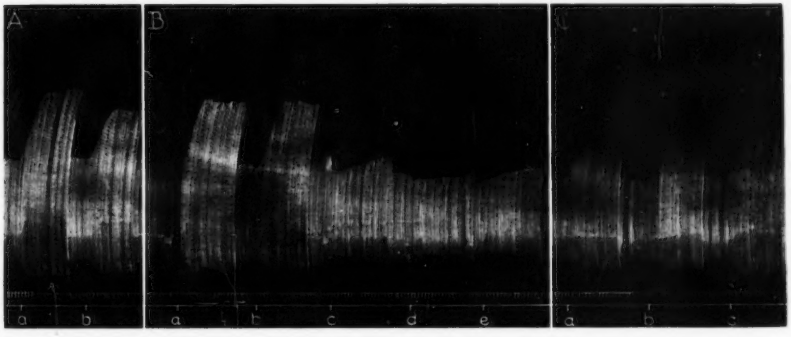

Fig. 6A. Hypodynamic frog heart. At *a*, 0.01 γ adrenaline (adr.); at *b*, adr. 0.005 γ.

B. At *a*, cat's superior mesenteric plexus, with artery, submerged in Ringer, one end stimulated 10 minutes; at *b*, same as *a*, but dialyzed; at *c*, vagus of cat, one end submerged in Ringer, other stimulated 10 minutes; at *d*, same as *c*, but dialyzed; at *e*, superior mesenteric artery of cat freed from nerve fibers, stimulated in Ringer 10 minutes.

C. At *a*, cat's superior cervical ganglion in Ringer, stimulated 10 minutes; at *b*, preganglionically denervated superior cervical ganglion of cat in physostigmine-Ringer, stimulated 10 minutes; at *c*, adr. 0.01 γ.

in vitro. However, when the solution in which such ganglia were stimulated was tested on a hypodynamic frog heart a typical adrenaline-like

effect appeared (fig. 6Cb). In cats the vagi were cut below the diaphragm. After complete degeneration of the vagus fibers the postganglionic sympathetic strands along the superior mesenteric artery were isolated. In some cases the nerves were excised together with the artery to avoid the damage of the fibers. The nerves were stimulated in 1 cc. physostigmine-Ringer solution. These solutions showed a strong adrenaline-like positive effect on the hypodynamic frog heart (fig. 6B).

In order to eliminate the presence of acetylcholine and thus detect only the possible positively-acting substances, cholinergic and mixed nerves (vagus and sympathetic of the neck, phrenic, sciatic, superior mesenteric plexus, superior cervical ganglion) were stimulated in Ringer-solution without physostigmine, and the solutions were tested on hypodynamic

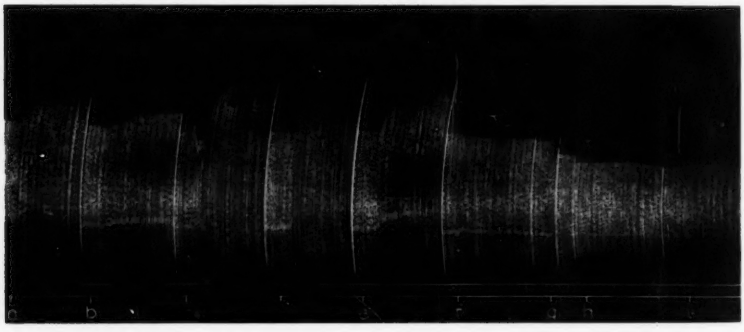

Fig. 7. Hypodynamic frog heart. At *a*, sciatic of cat, stimulated in Ringer 10 minutes and dialyzed; at *b*, adr. 0.0005 γ; at *c*, superior mesenteric plexus of cat, stimulated 10 minutes in Ringer; at *d*, adr. 0.01 γ; at *e*, same as *c*, but dialyzed; at *f*, same as *e* after heating at boiling point for 1 minute; at *g*, heart washed with ergotoxine-Ringer during 2 hours; at *h*, adr. 0.01 γ; at *i*, same as *c*.

frog hearts. The mesenteric plexus and superior cervical ganglion (with postganglionic fibers) liberated always a substance which had a strong positive inotropic and chronotropic adrenaline-like effect on the hypodynamic frog heart (fig. 7c). Mixed nerves, like the sciatic, liberated also this substance but in less quantity than the mesenteric plexus (fig. 7a).

Usually when any isolated nerves are stimulated there appear in the solution some substances other than adrenaline which are able to increase the inotropism of the hypodynamic frog heart. Even without any stimulation some positively acting substances appeared in the solution. Their action is different from the adrenaline-like effect, however, because the latter appears in a few seconds and disappears relatively soon after washing with Ringer, whereas the non-specific effect mentioned above appears later and takes a long time to disappear after washing. Heating the solution

does not destroy this effect, but evaporating and ashing the solution eliminates it. The substances involved are therefore thermolabile and of organic nature—possibly some proteins and lipoids from the blood and nerve. These substances are known to have a strong positive effect on the hypodynamic frog heart (for literature see Lissák, 1936; Lissák and Hoyos, 1936). If the solutions are tested for acetylcholine on the maximally beating normal frog heart, the lipoids and proteins are without influence. In tests for adrenaline on a hypodynamic frog heart, however, these substances confuse the observations. Their large molecules can be excluded by dialysis.

When adrenergic elements are stimulated in Ringer solution and the solution is dialyzed, the dialysate has adrenaline-like positive inotropic and chronotropic effects on the hypodynamic frog heart (figs. 6, 7). On the other hand, no adrenaline-like substance was detected when the nerve was submerged for the same time (10 min.) without any stimulation, and the solution dialyzed. With similar procedures carried out on cholinergic nerves no evidence of an adrenaline-like substance was obtained (fig. 6Bc, d).

Control experiments showed that the substance liberated when adrenergic fibers are stimulated *in vitro* has properties similar to adrenaline. The substance is dialyzable, thermolabile (fig. 7f), oxidizable, and its positive inotropic and chronotropic effects on the hypodynamic frog heart are abolished by ergotoxine (fig. 7h, i).

Discussion. The experiments reported in section A show, in confirmation of previous reports, that somatic motor, parasympathetic and preganglionic sympathetic nerves, and also sympathetic ganglia, when stimulated *in vitro*, liberate acetylcholine in different amounts. Gaddum, Khayyal and Rydin (1937) stated that acetylcholine is liberated from nerve trunks only at the stimulated region and only upon the passage of excessively strong stimuli, when visible desiccation and discoloration occurred, due probably to heating of the nerve. In view of the control experiments illustrated in figure 4 and of the precautions mentioned under "method" (p. 263), it is obvious that acetylcholine may be liberated from cholinergic nerves, stimulated in satisfactory physiological conditions. The evident ease with which such liberation took place in the present experiments makes difficult an explanation of the failure of Gaddum, Khayyal and Rydin (1937) to repeat the experiments of Binet and Minz (1936).

In his interesting study on the release of acetylcholine from perfused superior cervical and nodosum ganglia Lorente de Nó (1938) reported that such a release took place; not only when the sympathetic preganglionic fibers were stimulated but also when vagal or sympathetic postganglionic elements were activated. The present data readily account for the ap-

pearance of the substance upon stimulation of either preganglionic sympathetic or vagal fibers; they do not account, however, for the release of acetylcholine by stimulation of adrenergic postganglionic elements (see fig. 6B, p. 267).

The failure to find the liberation from cholinergic nerves of a substance which sensitizes the frog heart to acetylcholine, or to observe such an action from vitamin B_1 (fig. 5), is in contrast with the observations of Binet and Minz (1934, 1936). This difference is probably due to the different test objects used. Since this research was completed Glick and Antopol (1939) have shown that vitamin B_1 (thiamine) has an anticholinesterase action. Inhibition of the cholinesterase of serum, however, was obtained only "with concentrations of thiamine in excess of those known at present to occur within living organisms."

Physostigmine does not significantly sensitize the isolated frog heart to acetylcholine. It appears usually to prolong the negative inotropic effect, but does not always increase it (Loewi and Navratil, 1926). On the other hand, on leech muscle, as is well known from Führer's (1918) observation, the sensitizing effect of physostigmine is very great.

The experiments outlined in section B present evidence that postganglionic sympathetic fibers, sympathetic ganglia and mixed nerves like the sciatic, which contain postganglionic sympathetic fibers, liberate adrenaline in different amounts when stimulated *in vitro*. The control experiments show that when using the perfused frog heart as test object care must be taken to eliminate from the solutions other positively acting substances which confuse an adrenaline-like effect. The results of Calabro (1933), Rossine (1936a and b), Babsky and Kisliuk (1938), who found an adrenaline-like effect by stimulating *in vitro* the vagus and the sympathetic trunk of dogs and rabbits, are probably due to such substances.

The data presented in sections A and B lead to the inference that the production of acetylcholine or adrenaline by cholinergic or adrenergic fibers, respectively, and the liberation of these substances during nervous activity, is not limited to the nerve endings but extends to the entire axons, perhaps to the entire neurones. The functions of these active substances when liberated by the nerve endings at the effectors are now well known. Future studies will determine whether their presence in the nerve fibers is important for the functions of the fibers or is merely incidental to excitability and conduction.[2]

[2] Since this paper was sent to press H. C. Chang, W. M. Hsieh, L. Y. Lee, T. H. Li and R. K. S. Lim have published a similar study (Chinese J. Physiol. **14:** 19–27, 1939). Their results are in fundamental agreement with the present data. Chang *et al.* report the presence of acetylcholine in the hepatic nerves. This is not surprising since the nerves contain both sympathetic adrenergic postganglionic fibers and parasympathetic cholinergic elements.

SUMMARY

Isolated nerves of cats and frogs were stimulated *in vitro* in Ringer solution containing physostigmine (1:100,000), or in simple Ringer. After dialysis the solutions were tested on the isolated frog heart. Parasympathetic nerves and ganglia, and sympathetic preganglionic nerves and ganglia liberated acetylcholine in different amounts (section A, fig. 2).

Nerve trunks composed only of adrenergic fibers and preganglionically denervated superior cervical ganglia, when stimulated, liberate no acetylcholine but an adrenaline-like substance (fig. 6, 7).

Mixed nerves, such as frog and cat sciatics, and innervated sympathetic ganglia, release, when stimulated, both acetylcholine and adrenaline (figs. 1, 3, 6 and 7).

This release occurs in satisfactory physiological conditions—i.e., the nerves are not damaged by the procedures followed (fig. 4).

It is concluded that cholinergic nerve fibers liberate acetylcholine and adrenergic fibers liberate adrenaline during stimulation.

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THE VENTRICULAR RATE IN FARADICALLY MAINTAINED AURICULAR FIBRILLATION: AN INDEX OF A-V CONDUCTIVITY[1]

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Received for publication May 29, 1939

In studies intended to investigate the action of the chemical mediators of autonomic nerve action, attention as far as the heart is concerned has been directed to the effects on rate of discharge of the sinus pacemaker, on the magnitude of contraction and on the duration of the refractory period (Cannon (1, 2), Rosenblueth (3), Brown and Eccles (4), Gilson (5–8), Ashman (9) and Eccles (10)). It seemed to us that the conductivity through the A-V junctional tissue might serve in a similar rôle. While the measurement of A-V conductivity by conduction time for the transmission of sinus impulses or induced auricular extrasystoles is useful, it is not satisfactory in detecting small conduction changes and in following the time sequence of rapid changes. A continuous analysis of A-V conductivity can be obtained by following the ventricular rate of beats of supraventricular origin while the auricles are kept fibrillating by continuous faradic stimulation. Under such circumstances the bombardment of the A-V junctional tissues is sufficiently excessive to permit continuous evaluation of even small changes in its conductivity.

In the present report we are presenting our data on the effects on A-V conductivity measured in this way of 1, vagal and sympathetic stimulation, alone and in combination; 2, adrenalin and cholinergic drugs, and 3, asphyxia.

PROCEDURE. Fifteen dogs anesthetized with nembutal (25 mgm. per kilo intravenously) were used. The chest was opened and artificial respiration instituted. The auricles were kept in fibrillation by stimulation with a Harvard inductorium by means of small hook electrodes inserted through a slit in the pericardium into the right atrial wall. The current strength was minimal for production of constant auricular fibrillation.

Both vagi were cut high in the neck and the peripheral end of one or the other was faradically stimulated with non-polarizable electrodes by means of a Harvard inductorium. The current strength was adjusted to that level which produced definite heart rate changes prior to the induction of

[1] Aided by the A. D. Nast Fund for cardiac research.

auricular fibrillation. The right and left stellate ganglia were stimulated in a similar manner.

The actions of acetylcholine, mecholyl (acetyl-*B*-methylcholine chloride), adrenalin and asphyxia were determined in the completely denervated heart. The vagi were previously cut and the stellate and upper five thoracic sympathetic ganglia and all their visible branches on both sides were resected. The drugs were given intravenously. Asphyxia was produced by stopping the artificial respiration.

The actions of nerve stimulation, drugs and asphyxia were analyzed from records obtained with lead II of the electrocardiograph. The time of stimulation was recorded with signal magnets connected in series with the inductoria used to stimulate the nerves. The time of injection of the drugs and of asphyxia was recorded in a similar manner. The zero time in each experiment was taken as the time of onset of stimulation or injection, or cessation of respiration.

RESULTS. *1. Effect of vagal stimulation.* Stimulation of the peripheral end of either cut vagus for 10 to 25 seconds produced a marked and almost immediate depression of A-V conductivity (figs. 1B, 2A, 3A). This is in accord with previous observations (11–16). No significant differences between stimulation of the right and left vagi were seen. This is in agreement with Robinson's findings (11) but not with those of Cohn (15) and Cohn and Lewis (16) who reported that the left vagus had a greater effect. Variations in effect may be expected since Bachman (17) has reported a variation in the distribution of the nerve fibres to the S-A node in different individuals and species.

When vagal stimulation was maintained, the conductivity of the A-V tissues showed a gradual return toward normal (fig. 3A). The return of A-V conductivity toward normal following cessation of stimulation was rapid and began almost at once. Following the longer stimulation, a mild transient post-stimulatory overswing was frequently but not constantly manifested (fig. 3A).

2. Effect of sympathetic stimulation. Stellate ganglion stimulation was effective in enhancing A-V conductivity. This is in accord with the observations of Winterberg (18) and Rothberger and Winterberg (19). We found the right and left nerves equally effective. Vagal stimulation differed in its effects from sympathetic stimulation in four ways: the effect was opposite, the magnitude of the effects of sympathetic stimulation was less than with vagus stimulation, a lag of 1 to 5 seconds was found before the effects of sympathetic stimulation became apparent and a long persistence of the augmentory action was observed without any overswing on returning to normal (figs. 1A and 2B).

3. Effect of vagus and sympathetic nerve section. The results of nerve stimulation indicated poor vagal tone in these animals when the vagi

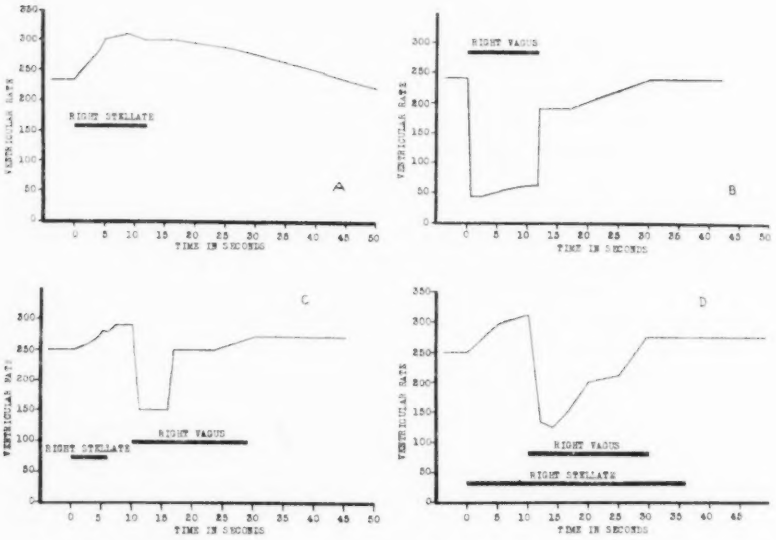

Fig. 1. Consecutive experiments on one dog. Auricles fibrillating, vagi cut. Heavy black lines indicate duration of stimulation. Described in text.

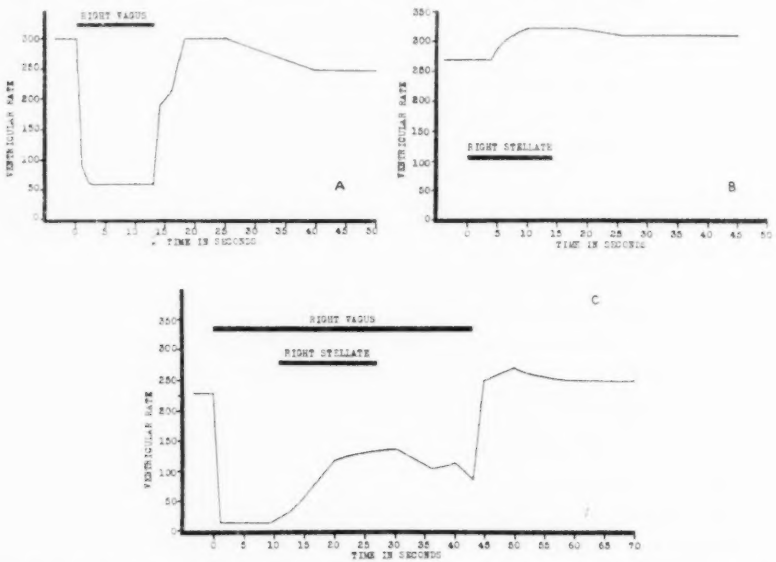

Fig. 2. Consecutive experiments on one dog. Auricles fibrillating, vagi cut. Heavy black lines indicate duration of stimulation. Described in text.

were intact, since bilateral vagus section had little effect on A-V conductivity. The low vagal tone may have been due to the barbital anesthesia (20). In contrast, however, removal of the sympathetic nerves supplying the heart caused a definite depression of A-V conductivity, the ventricular rate changing from about 250 to about 150 beats/min.

4. Effect of combined stimulation of vagus and sympathetic nerves. The effect on A-V conductivity of simultaneous or sequential stimulation of the vagus and sympathetic nerves was studied in 5 animals. Selected typical curves are shown in figures 1C, 1D, 2C, 3B. It is apparent that, taking into account the differences in latency, persistence, overswing and magnitude of the responses to stimulation of the two nerves, the effects are roughly additive algebraically. Thus the depression of A-V conductivity upon vagus stimulation was diminished in intensity if it was immediately preceded by stimulation of the sympathetic nerves (fig. 1C and 1D). The vagal escape appeared sooner and was more marked.

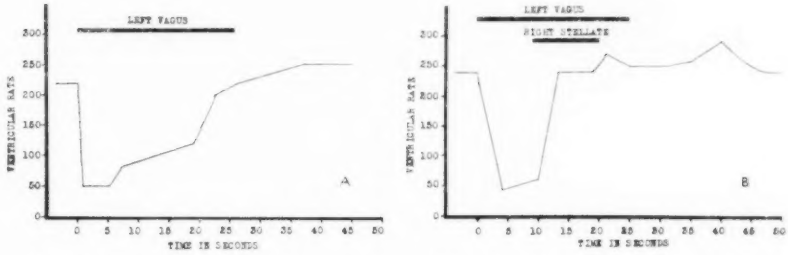

Fig. 3. Consecutive experiments on one dog. Auricles fibrillating, vagi cut. Heavy black lines indicate period of stimulation. Described in text.

In addition, the A-V conductivity at the end of the period of vagus stimulation, following sympathetic nerve stimulation, was above the level established before either of the nerves was stimulated (fig. 1C and 1D) while in this same animal, stimulation of the vagus alone caused the A-V conductivity following stimulation to remain temporarily below the control level (fig. 1B).

Again, in fig. 2C, the superposition of the effect of sympathetic stimulation (fig. 2B) upon the effect of vagal stimulation (fig. 2A) is readily observed in the lessening of the vagal depression of A-V conductivity during and following sympathetic stimulation. The magnitude, latency and persistence of this sympathetic stimulation has the characteristics of the effects of sympathetic stimulation alone.

In figure 3B, the neutralizing effect of sympathetic stimulation upon the vagus depression of A-V conduction is striking as can be seen by comparing this record with the record of vagus stimulation alone (fig. 3A).

5. Effect of adrenalin. Adrenalin (0.33 cc. of a 1/1000 solution) constantly produced a definite and rapidly appearing enhancement of A-V conductivity (fig. 4D). The persistence of this effect and the lack of overswing resemble the action of sympathetic nerve stimulation. The greater response obtained is doubtless due to a lower level of A-V conductivity existing in the denervated heart. 933 F (piperidomethyl-3-benzodioxane) in doses of 5 mgm. intravenously and ergotamine tartrate in amounts of 0.2 to 10 mgm. intravenously had no appreciable effect on the adrenalin response.

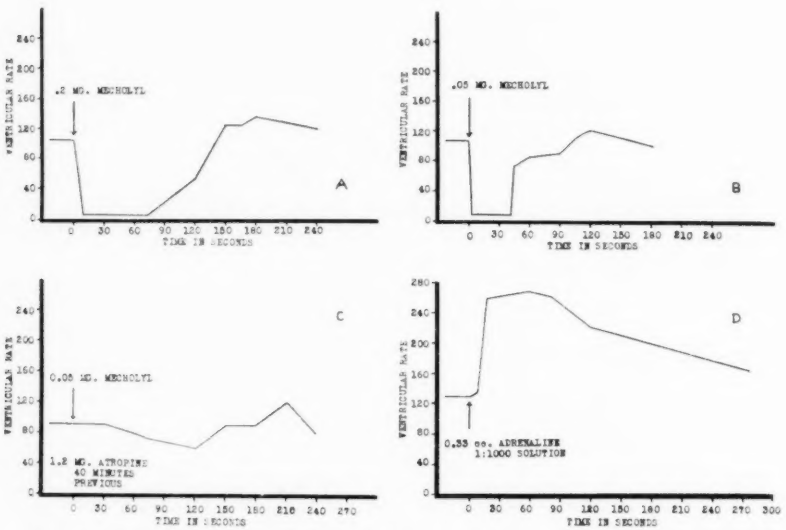

Fig. 4. Denervated heart, auricles fibrillating. A, B and C represent experiments at approximate half-hour intervals on the same dog. D represents a similar preparation on another dog. Note the slower control ventricular rate in the denervated preparations. Described in text.

6. Effect of cholinergic drugs. Intravenous infusion of acetylcholine or mecholyl produced immediate and marked depression of A-V conductivity. The effect varied with the amount of drug injected. In doses of 0.05 mgm. intravenously, mecholyl produced an immediate marked depression of A-V conductivity lasting about 25 seconds (fig. 4B). Mecholyl in doses of 0.2 mgm. intravenously produced complete heart block lasting about 70 seconds (fig. 4A). The restoration of A-V conductivity following its depression was fairly rapid, but was usually accompanied by a temporary overswing. Acetylcholine in amounts of 25 to 100 gamma intravenously produced a similar depression of A-V conductivity (fig. 5A).

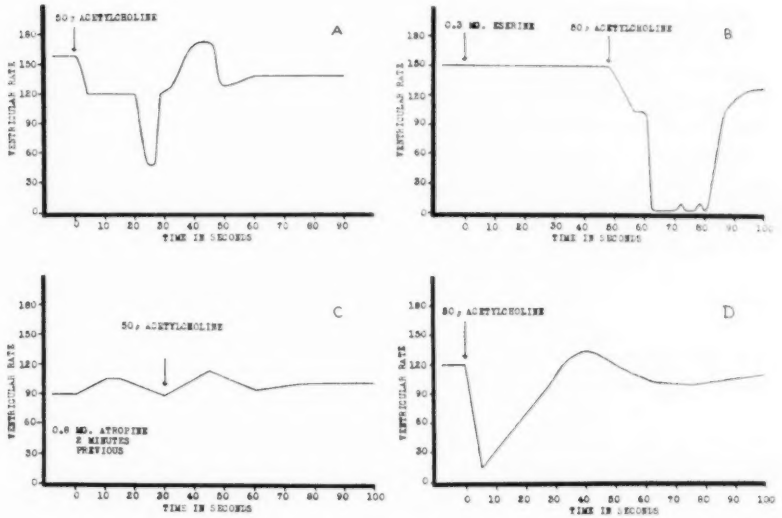

Fig. 5. Denervated heart, auricles fibrillating. Consecutive experiments at approximate half hour intervals on one dog. Described in text.

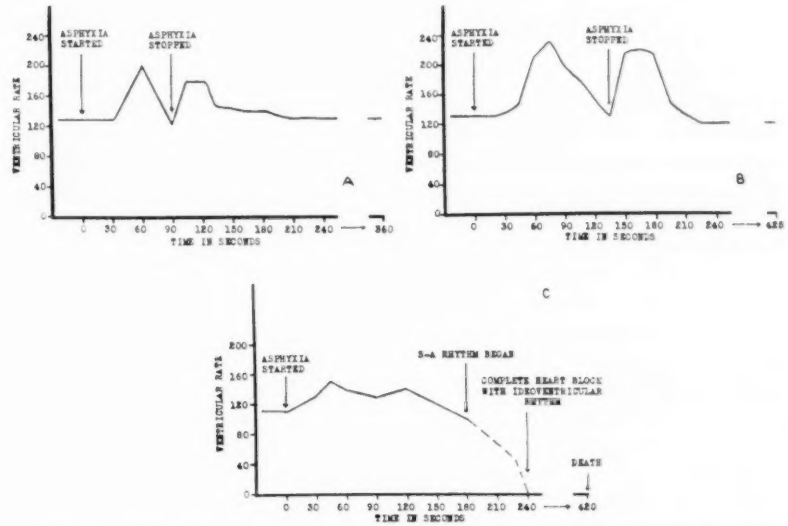

Fig. 6. Denervated heart, auricles fibrillating. A, B and C represent separate experiments on different dogs. Described in text.

Previously administered atropine sulphate (0.7 to 1.2 mgm. intravenously) diminished or abolished the depression of A-V conductivity produced by the cholinergic drugs (fig. 4C and 5C). The atropine effect varied directly with the quantity administered and inversely with the time elapsing between its administration and the administration of the cholinergic drug. In sufficient doses, (0.8 mgm.) atropine completely abolished the effect of acetylcholine (50 gamma dose) (fig. 5C).

Eserine sulphate (0.3 to 1 mgm. intravenously) augmented both the degree and duration of the acetylcholine depression (fig. 5B).

7. *Effect of asphyxia.* Typical experiments of asphyxiation in denervated hearts are shown in figures 6A, B and C. In the early stages asphyxia enhanced A-V conductivity; in the more advanced stages it depressed A-V conductivity until eventually complete A-V block appeared. When respiration was resumed, a period of enhanced A-V conduction appeared before normal A-V conduction was regained. This early improvement of function seen before the depression occurred resembles other actions of asphyxia, viz., its action on blood pressure. Lewis (21) noted only a gradual slowing of ventricular rate under similar experimental conditions in the innervated heart. As yet we have not investigated the mechanism of this primary enhancement.

Discussion of results. The resemblances between adrenalin action and sympathetic stimulation and between cholinergic drug and vagus action on A-V conductivity suggests that the A-V conductivity can be modified by the presence and action of chemical mediators. The greater action of adrenalin in the denervated heart as compared with that of sympathetic nerve stimulation superimposed on its tonic action when the sympathetics are intact appears to be connected with the basal A-V conductivity in the two conditions. Thus, as the upper limit of the conducting ability of the A-V junctional tissues is approached, reduction in the ability to augment A-V conductivity occurs. This may also explain why depression was so much more prominent than augmentation in the experiments in which the sympathetic nerves were intact.

The overswing following the cessation of the longer vagus stimulation is probably a compensatory mechanism associated with the extremely slow heart rate and may involve reflexes over the sympathetic nerves to the heart and the liberation of adrenalin. The latter mechanism may explain the overswing following the administration of cholinergic drugs since it is known that they stimulate the intact sympathetic nerves to the adrenals, presumably by acting at the preganglionic nerve synapses within the sympathetic ganglia.

The characteristics of the action of the sympathetic and vagus nerves on A-V conduction as regards the direction, latency and persistence of

action resembles their effects on other properties of the heart (cf. Kuntz (22)).

Our method is applicable to the evaluation of other drugs and procedures. Information thus obtained might be useful in supplying new drugs and procedures which can act, on the one hand, to overcome functional A-V block clinically, and, on the other hand, to depress A-V conductivity when the frequency of auricular impulses is excessive as in auricular fibrillation and flutter. Our results with nerves and drugs which are in accord with clinical knowledge (23–26) favors such application.

SUMMARY

1. With the A-V tissues subjected to a constant bombardment of impulses arising in the auricle by faradically maintained auricular fibrillation, the ventricular rate (of beats of supraventricular origin) was used as an indicator of A-V conductivity.

2. Using this method, the following results were obtained on A-V conductivity: *a*, Vagal stimulation produced a depression. *b*, Sympathetic stimulation produced an enhancement. *c*, Simultaneous or successive stimulation of the vagi and sympathetics were algebraically additive. *d*, Acetylcholine and mecholyl produced a depression which was prevented by atropine and increased by eserine. *e*, Adrenalin produced an enhancement. *f*, Asphyxia produced a primary enhancement followed by a depression. These effects occurred in reverse order on recovery from asphyxia.

3. The differences in direction, lag and persistence of the effects of vagus and sympathetic nerve stimulation are similar to the known differences in the action of these nerves on other cardiac properties.

4. The resemblance of the effects on A-V conductivity between vagus stimulation and cholinergic drug action on the one hand, and the resemblance between the effects of sympathetic stimulation and adrenalin, on the other, lend support to the view that the action of the nerves on A-V conductivity is mediated chemically.

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STUDIES ON THE OXYGEN DEBT OF FROG TISSUES

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Received for publication June 1, 1939

It is clear from the work of several investigators that frog skeletal muscle compared with nerve, skin, or cardiac muscle possesses a peculiarly effective mechanism for repayment of an oxygen debt contracted by a period of anoxia (*cf.* Fenn, 1930a, b; Brody, 1934). Earlier studies on skeletal muscle were made by Meyerhof (1920) and on nerve by Gerard (1927). Although no fundamental objections to this conclusion have appeared, the wide fluctuations in the extent of repayment of the debt in different samples of tissue, especially skeletal muscle, and inherent difficulties in the technique used made confirmation and extension of the experiments advisable. This has been done by a more refined technique than that used in earlier experiments, and the series has been extended to liver, kidney, stomach and intestine in addition to skeletal and cardiac muscle, peripheral nerve, and skin. The unique ability of skeletal muscle to pay off an oxygen debt persists in the more extended list of tissues and a possible correlation with the phosphocreatine content of the tissues has been studied.

METHOD. Specially designed differential volumeters were employed using the same principle as that employed by Brody (1934) and Fenn (1934). The two apparatuses are illustrated in figure 1. The tissue can be placed in a large hollow stopcock, *s*, connected to or disconnected from the main chamber, *c*, at will. After a control period in oxygen the whole apparatus can be flushed with nitrogen. Then, after a few readings to assure that the oxygen was completely removed, the tissue can be shut up in the stopcock, *s*, for the balance of the anaerobic period while the main chamber, *c*, is filled with pure oxygen. The rate of oxygen consumption immediately after the anaerobic period is then measured by reconnecting the stopcock to the main chamber. Apparatus A was soon supplemented by and in some cases replaced by apparatus B, the advantages of which will be explained in connection with the experimental results.

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The "real" oxygen debt was estimated by assuming that the pre-anaerobic rate of oxygen consumption persisted as an equivalent need for oxygen during the anaerobic period. The repayment of the debt was then the ratio of the extra oxygen consumed (expressed as a percentage) after anaerobiosis to the amount of oxygen missed.

The maintenance of a steady base line is of course important for such calculations. For this reason in the series on skeletal muscle the dissection was always made the night before an experiment and the muscles equilibrated at about 5°C. This yields a steady resting rate of oxygen consumption (Stannard, 1939). The other tissues were used an hour or two after dissection since the rate of oxygen consumption was not made any more stable by longer equilibration, and was sufficiently constant for our purposes.

The nitrogen was freed of oxygen by passage through a train of three bottles containing Fieser's solution, thence through lead acetate, and finally over hot copper. No rubber connections were used at the final end of the train. It was found that more complete anaerobiosis could be obtained by this method with the apparatus in hand than by passing hydrogen over hot platinized asbestos. This advantage was offset somewhat by the fact that nitrogen and oxygen do not have the same solubilities in water while hydrogen and air do (Fenn, 1930a). However the net change in volume on exchanging oxygen for nitrogen in the gas space is calculable, and was eliminated in theory by making the ratio of gas space to fluid volume always the same within the stopcock and the main chamber.

The experimental temperature was 22°C. For the composition of the Ringer's solution see Stannard (1938).

Skeletal muscle. The first experiments on skeletal muscle were carried out in apparatus A. The muscles were attached to glass rods and placed within the stopcock over a few drops of Ringer's solution. The KOH was placed in the main chamber. However, the muscles could not be maintained in good condition by this method and as rigor approached the baseline steadily rose (Fenn, 1930b) making calculation of the repaid O_2 debt uncertain. The technique was modified by placing the muscles in the main chamber immersed in Ringer's solution and the KOH on a ring of filter paper at the tip of the ground glass joint to the capillary. The oxygen for measurement of the recovery rates was then flushed through the stopcock, *s*, after turning this away so as to close it off from the main chamber, *c*. The initial and final rates of O_2 uptake were constant and nearly identical by this method. However, slow diffusion of gas between the stopcock and bottle when the two were reconnected presented a disadvantage. In addition the small amount of oxygen contained within the stopcock since it was diluted by the larger volume of nitrogen in the main chamber on establishing reconnection, was found in certain cases to present

insufficient oxygen tension to support the maximum rate of oxygen consumption of the tissue.

For these reasons apparatus B was designed. In this model the hollow stopcock, *s*, is equivalent to a central cylindrical space surrounded by the main chamber, *c*. The stopcock can be filled from above by removal of the upper stopper and the tissue immersed in Ringer's at the bottom. A ring of filter paper soaked with KOH was placed at the tip of the upper stopper for absorption of CO_2. The steps in an experiment were the same as described previously. It was found in control experiments with this apparatus that equilibrium between the two chambers ensued very quickly on establishing connection between them. Also since the volume of oxygen present in the main chamber was large compared with the volume of nitrogen in the stopcock there was no difficulty in maintaining sufficient oxygen tension during the recovery period.

The experiments on muscle are illustrated in table 1. In series A the stopcock was left connected to the bottle during the anaerobic period for measurement of any residual oxygen consumption that might occur. Nitrogen was not passed through the capillary (i.e., the index drop was not broken) and it was found that the small amount of oxygen left in the capillary was indeed sufficient to support a small apparent O_2 consumption. The figures for the O_2 missed and thus the *per cent* of the debt repaid were corrected for this small residuum. In series B the conditions were made completely anaerobic by passing nitrogen through the capillary and then reassembling the index drop. It will be noted that the *per cent* repaid is considerably lower in this series. Possibly there is a significant difference between complete anaerobiosis and the presence of a small amount of oxygen in determining the subsequent ability of the muscle to repay an oxygen debt. However, series C was similarly carried out under conditions of as complete anaerobiosis as possible, yet the repaid debt amounts to 68 per cent of that incurred. Series C involved a fresh lot of frogs while series B employed frogs which were badly emaciated. It is possible that the inability of the muscles in series B to repay an O_2 debt is related to this fact.

The average figures for series C, the best series, show that frog skeletal muscle repays approximately 70 per cent of the O_2 missed during anaerobiosis, in precise agreement with the best figures of Fenn. The refinements in technique have served, however, to reduce the range of variation seen in his experiments and in no case was there any tendency for the muscles to consume an extra amount of oxygen greater than the amount missed. This phenomenon complicated many of Fenn's experiments. It seems likely that this figure represents the true figure for isolated skeletal muscle of the frog determinable by this method. The "recovery" is apparently never quite complete. Whether this represents the true

phenomenon or some error in determining the amount of debt incurred should be determined in the future (see discussion).

TABLE 1

Repayment of oxygen debt in skeletal muscle

WEIGHT OF TISSUE	INITIAL RATE	FINAL RATE	TIME IN N_2	O_2 MISSED	O_2 REPAID	
			Series A			
grams	*mm.³/g./hr.*	*mm.³/g./hr.*	*min.*	*mm.³*	*mm.³*	*per cent*
0.329	39	45	220	31.8	31.8	100
0.330	38	35	224	39.0	40.0	103
0.345	39	44	195	30.6	26.1	85
0.226	51	55	138	16.0	15.0	94
0.217	40	38	190	9.9	9.0	91
0.252	27	28	200	15.0	14.6	97
0.306	37	37	200	29.8	19.0	64
Average						91
			Series B			
0.161	32	32	142	11.8	4.7	40
0.156	39	38	195	20.2	9.0	45
0.298	25	26	180	21.4	8.6	40
0.224	39	35	200	29.2	13.9	48
0.214	51	45	235	43.0	22.5	52
Average						45
			Series C			
0.224	37	37	185	24.9	15.5	62
0.230	35	34	180	29.3	15.5	53
0.124	35	35	270	19.5	18.8	95
0.266	39	39	187	33.0	21.1	64
0.265	40	42	180	31.0	25.8	83
0.271	38	38	150	25.9	14.4	56
0.276	47	47	150	32.5	18.1	56
0.262	49	46	263	46.1	33.5	73
0.278	36	34	262	43.7	28.9	66
0.226	45	41	250	46.0	23.6	51
0.245	46	38	280	46.3	24.4	53
0.283	39	39	155	29.0	25.3	88
0.275	34	32	200	29.5	23.5	80
0.238	36	35	150	27.4	21.2	77
0.226	40	39	180	30.8	20.2	66
Average						68

Other tissues. In table 2, experiments similar to those described for muscle (series C) are summarized for kidney halves, ventral and dorsal

TABLE 2

*Repayment of oxygen debt in other tissues**

TISSUE	WEIGHT OF TISSUE	INITIAL RATE	FINAL RATE	TIME IN N_2	O_2 MISSED	O_2 REPAID	
	grams	*mm.³/g./hr.*	*mm.³/g./hr.*	*min.*	*mm.³*	*mm.³*	*per cent*
Kidney	0.083	309	207	159	68	0	0
	0.146	284	246	162	107	0	0
	0.087	289	156	192	76	0	0
	0.131	192	185	125	66	0	0
	0.060	309	196	120	42	0	0
Liver	0.140	252	169	161	96	0	0
	0.145	222	130	156	84	0	0
	0.140	169	139	105	42	0	0
	0.151	185	159	120	56	0	0
	0.090	350	283	169	88	0	0
	0.130	264	129	180	102	0	0
	0.090	380	200	134	75	0	0
Heart	0.113	108	108	165	50.2	1.2	2
	0.102	186	186	159	50	0	0
	0.158	161	161	110	28	1.3	5
	0.113	145	115	230	57	0	0
Average							1.8
Nerve	0.060	48	46	165	10.2	0.7	7
	0.090	24	24	165	6.3	0	0
	0.076	78	69	168	15.9	0	0
	0.045	40	42	205	6.1	0†	0†
Average							1.8
Skin	0.112	110	103	188	38.4	5.8	15
	0.098	164	143	160	43.1	2.1	5
	0.128	171	158	113	41.4	0.7	2
	0.139	150	144	112	38.9	4.6	12
Average							8.5
Stomach	0.164	109	87	147	43.8	0	0
Intestine	0.200	127	112	121	51.3	0	0
	0.135	204	195	152	69.6	0	0

* In many cases where the repaid debt is recorded as zero it will be noted that the final rate is lower than the initial rate. Thus the calculated debt repaid would be actually a negative quantity. However since these rates never returned to the initial level, negative figures would be erroneous and meaningless. They are recorded as indicating no tendency to repay the debt. (See discussion in text.)

† Actually the rate was about 5 per cent higher after anaerobiosis and remained so for the balance of the experiment.

skin, intestine, stomach, liver slices, quiescent strips of ventricle, and isolated sciatic nerves. It will be noted that in no case is any appreciable fraction of the O_2 missed during anaerobiosis regained by means of an "extra" oxygen consumption during the subsequent period in oxygen. The figures for nerve are enough lower than those reported by previous investigators to indicate that this tissue does not repay its debt at all, rather than only partially. The data for skin, while lower than those reported by Fenn (1930a), still indicate a possibly significant ability to repay an O_2 debt.

It will be noted that especially in the experiments with liver and kidney the final rate of oxygen consumption is consistently below the initial rate. Skin, stomach, and intestine also exhibited this decline in rate but to a lesser extent. Obviously calculation of the O_2 repaid on the basis of the initial rate would yield a negative value, i.e., a negative O_2 debt. Therefore, controls were necessary, especially in the case of liver and kidney, to determine to what extent this decline would have occurred without a period in nitrogen, and especially whether or not the rate after anaerobiosis was higher than the control after the same period in oxygen, which would indicate repayment of the O_2 debt. Actually, the reverse was true for liver and kidney, viz., the rates for tissue kept in oxygen continuously fell only slightly with time and agreed reasonably well with the initial rates shown in table 2. In the other tissues where a slight decline in rate after anaerobiosis occurred it was no greater than normally seen without a period in nitrogen. Thus liver and kidney exhibited a marked fall in rate of O_2 uptake due to the anaerobiosis. Although there is no apparent correlation between the length of the period in nitrogen and the subsequent rate of oxygen consumption it seems possible that lack of oxygen was damaging to liver and kidney. Skin, nerve, heart, intestine, and stomach, while exhibiting no marked tendency to repay an O_2 debt did not appear to be damaged by anaerobiosis at least with respect to the ability to consume oxygen at the same rate afterwards as tissues kept in oxygen continuously.

Phosphocreatine. In his study of frog cardiac muscle Brody (1934) compared the relative importance of phosphocreatine in the buffering process of skeletal and cardiac muscle during anaerobiosis. The method of Meyerhof and Lipmann (1930) was employed in which the absorption of carbon dioxide in a CO_2-bicarbonate system was taken as a measure of the relative amount of phosphocreatine breakdown. Brody found that in cardiac muscle phosphocreatine played a much less important rôle than in skeletal muscle. This agrees with the known differences in phosphocreatine content, e.g., resting skeletal muscle contains about 65 mgm. per cent (Eggleton and Eggleton, 1929–30), and resting cardiac muscle only about 7.3 mgm. per cent (Clark, Eggleton, and Eggleton, 1931).

In the present experiments this comparison has been extended to most of the tissues studied. The results are presented in figure 2 as rates of CO_2 absorption or evolution against time. The gas phase contained 30 per cent CO_2 in nitrogen and the solution was Ringer-bicarbonate (0.02 N with respect to bicarbonate). The data are corrected for the initial burst of CO_2 absorption which represents saturation of the suspension fluid with the new gas mixture. This was evaluated in control experiments. It is apparent that in skeletal muscle the breakdown of phosphocreatine

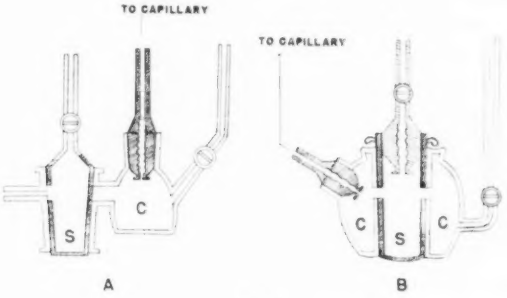

Fig. 1

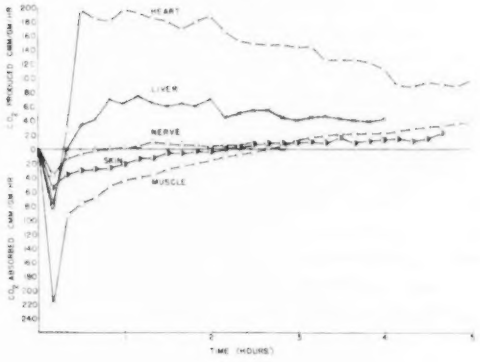

Fig. 2

is both more rapid and more extensive than in any of the other tissues tested. This agrees in general with the lower contents of this compound found in other tissues of the frog (Gerard and Wallen, 1929, for nerve; Clark, Eggleton and Eggleton, 1931, for cardiac muscle; Martino, 1928, for many other tissues). It is suggestive that skin, which of all the tissues other than muscle showed most consistently some slight tendency to repay the O_2 debt, indicates a larger relative breakdown of this substance during anaerobiosis. Among the experiments on liver there was some indication

that the damaging effect of anaerobiosis was less marked in those cases which showed a greater relative amount of phosphocreatine breakdown. Although more conclusive evidence is surely necessary it seems possible that the ability of various tissues to repay an oxygen debt may depend in some manner upon this portion of the glycogenolytic system either directly or through its buffering power.

Discussion. Nerve, cardiac muscle, intestine, stomach, kidney, and liver exhibit practically no tendency to repay the amount of oxygen missed during a period of anaerobiosis, while skeletal muscle in good condition repays nearly 70 per cent of the oxygen debt. This contrast between skeletal muscle and other tissues has been interpreted as indicating a "peculiarly effective mechanism" in skeletal muscle for withstanding periods of anaerobiosis and by inference the desirability that all tissues repay their oxygen debt. But only liver and kidney seemed to be actually damaged by the anoxia. The ability of peripheral nerve to recover functionally after a period in nitrogen is well known. Perhaps the presence of an oxidative reserve known for nerve (Gerard and Meyerhof, 1927), and heart (Fenn, 1934) accounts for this fact in the other tissues which apparently withstood the anoxia without at the same time contracting an oxygen debt. But another interpretation would be that it is unfair to assume that all tissues develop any considerable oxygen debt simply by missing oxygen. Even in the case of skeletal muscle there is no experimental proof that the "real debt" is correctly measured by the pre-anaerobic rate of oxygen consumption. For example, there is no convincing evidence that the energy release represented by the resting oxygen consumption represents the minimum for maintenance of the living state or indeed anything more than the amounts of enzyme and substrate reacting under basal conditions. There is little information in current ideas of the fundamental biochemical reactions underlying the metabolism of frog tissues to explain the unique properties of skeletal muscle in this respect. By emphasizing this unique position it is felt that the present investigations indicate the need for future examination of the underlying mechanism.

SUMMARY

Skeletal muscle maintains its unique position among frog tissues in its response to anaerobiosis. Nerve, cardiac muscle, intestine, stomach, kidney, and liver exhibit practically no tendency to repay the amount of oxygen missed during a period of anaerobiosis while skeletal muscle repays an average of 70 per cent of the oxygen missed. Skin exhibits a slight but consistent tendency in this direction. The more refined techniques used in this work have eliminated some inconsistencies found in previous data for muscle and indicate a sharper contrast between muscle and other tissues than earlier work. Liver and kidney seemed to be

damaged by the periods of anaerobiosis to which they were subjected while skin, nerve, cardiac muscle, and gut appeared to remain undamaged in spite of their failure to repay the O_2 debt. There is some indication that the property of repaying an O_2 debt is in proportion to the relative phosphocreatine content of the tissues.

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THE EFFECT OF pH CHANGES ON THE IN-VITRO O_2 CONSUMPTION OF TISSUES[1]

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Received for publication June 1, 1939

In the course of experiments in tissue respiration, we have as a matter of routine controlled the pH. On one occasion alkali was accidentally spilled into the medium of a respiration flask, but in spite of the very high resulting pH (above 11.5), the O_2 consumption in this flask was of the same order of magnitude as in the other flasks containing the same materials at pH 7.3. This artefact led us to an investigation of the effect of pH changes on O_2 consumption of tissues, especially beyond the physiological ranges, for which the data are not extensive. We have studied four guinea-pig tissues—liver, kidney, testis, and brain.

METHODS. The tissues were sliced freehand, except for testis, with suitable precautions as to thickness of slices, and the O_2 consumption determined in a Barcroft-Warburg differential manometer system, calculations being made on the basis of the dry weight. The slices were pooled and then distributed to the flasks containing on the one hand a medium at approximately pH 7.3 as a control, on the other, a medium at the desired pH. In the case of testis, the tissue was teased apart. In our earliest observations, we experimented with the use of Ringer's solution plus various buffer systems, such as Sörensen's phosphate mixture, borax-boric acid, and acetic acid-sodium acetate, because of the limitations in the buffering range of each buffer. This presented two difficulties: first, that the pH during the experiment tended to change markedly at the extremes of the range of each buffer; and 2nd, that the ions of the buffers introduced unknown variables in shifting from one buffer to another. We consequently in the experiments here reported used horse serum, made almost bicarbonate free by shaking with HCl at pH 6.0 to 6.5, and adding either HCl or NaOH to bring to the desired pH, so that the sodium and chlorine ions were the only complicating variables introduced directly to the medium.

The pH of the medium was determined with a glass electrode before the

[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

tissues were added—about a half-hour before the actual beginning of the experiment—and immediately following the experiment. On the acid side, the change during the experiment was negligible, the observed tendency to rise being rarely more than 0.1 pH; on the alkaline side, there was a tendency to fall, which at the higher levels was occasionally as high as 0.7 pH in the case of brain. (The arrows in the figures represent the direction and extent of the pH change during the experiment.) Observations indicated that while the slope of this fall was not a straight line, being greatest at the beginning, the error of assuming that it was, was not more than about 0.1 pH, consequently the average pH of the experiment was computed on this basis.

At pH's above 9.5, the serum itself began to consume oxygen, very little until about pH 10.5, when the O_2 consumption per cubic centimeter of serum was about 6 cu. mm. per hour, then at rapidly increasing rates, so that at pH 11.5 the serum consumed about 50 cu. mm. O_2 per cc. per hour. At the higher levels of pH a suitable correction was made for this spontaneous oxidation in the serum. The error of the determinations was no greater than 10 per cent and was usually less than 5 per cent.

The question arises as to whether the pH of the medium, which is what we were actually determining, really reflects the pH of the tissue. On that the evidence is meager. In the experiments of Fenn, Cobb, Hegnauer and Marsh (1934), a long soaking of nerve trunks in medium at pH 6.3 brought the calculated pH of the nerve to pH 6.8; and soaking in medium at pH 7.7 brought the nerve pH to 7.6. Our chief reason for believing that an equilibrium of hydrogen and hydroxyl ions existed throughout the experiment between the cells and the medium was the time factor. The experiments usually lasted one hour, with readings every fifteen minutes. While space forbids our presenting the detailed data, the evidence clearly indicated that such changes in O_2 consumption as occurred were quantitatively the same in each fifteen minute reading. If the tissues were gradually changing their pH during the experiment, a changing effect on the O_2 consumption would have been expected. Many years ago Warburg (1910) showed that the O_2 consumption of sea urchin eggs increased when the medium was made more alkaline, in spite of the fact that from staining reactions it appeared that the pH of the interior of the eggs was unchanged. He concluded that the physico-chemical reactions of the egg membrane must be the determining factor, in turn influenced by the pH of the medium. Such a possibility of course exists in the present experiments as well, though we must neglect it failing further evidence.

RESULTS. *1. Liver.* Koehler and Reitsel (1925) found the O_2 consumption of minced rabbit liver to fall off on either side of a fairly sharp optimum at pH 7.4. Zero was reached at about pH 5.5 and 9.7. Comel

(1928) studied the range from pH 3.0 to 9.1, the maximum QO_2 of minced frog liver being at pH 7.8. Mouse liver was found by Nomura (1937) to have a slightly increased O_2 consumption as the pH rose from 5.7 to 7.4; and Okabe and Kodama (1934) observed the same phenomenon in rabbit liver within the range of pH 6.7 and 7.7. Felix, Zorn and Dirr-Keltenbach noted a sharp optimum of O_2 uptake at pH 7.7 in minced liver to which tyrosin had been added. Beyond pH 6.8 and 8.2 there was practically no O_2 consumption. Reinwein and Singer (1927) found an optimum for guinea-pig liver at pH 8.4, with still a considerable O_2 consumption at pH 4.5 and 9.6. At the latter levels the Sörensen PO_4 mixture they used as a medium must have had practically no buffering power. All of these observers used a Ringer's medium, buffered except in the experiments of Koehler and Reitsel, who alone noted the final pH at the end of the experiment. In the case of the buffered media, different ions were of course introduced when the buffer system was changed.

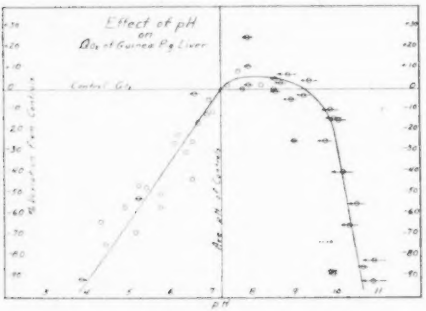

Fig. 1

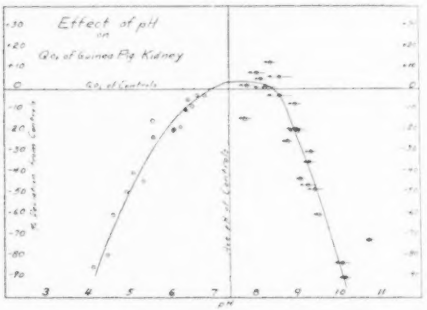

Fig. 2

Our own observations are summarized in figure 1. It will be seen that between pH 7.0 and 9.7, the consumption of O_2 by the tissue was constant within the limit of error. Below pH 7.0, the QO_2 fell as a straight line function of pH to zero at pH 4. On the alkaline side, the QO_2 began to fall rapidly at about pH 10, reaching zero at about pH 10.5. As the arrows in the figure indicate, there was no great deviation in pH during the experiment except at the higher alkaline levels.

2. Kidney. Observations on this tissue have been made by Nomura (1937) who observed an increased O_2 uptake by rabbit kidney as the pH was increased from 5.1 to 6.9; and by Okabe and Kodama (1934), whose results with kidney were similar to those they obtained with liver. Kisch (1932), using buffered Ringer's solution, studied the kidney QO_2 of rat, ox, and cat, between pH 6.5 and 8.5, the optimum QO_2 being in the neighborhood of 7.2–7.5. (Kisch's observations were made on a vari-

ety of tissues at these ranges, and in general, an optimum was found at physiological pH's.) In Reinwein and Singer's (1927) experiments, an optimum was observed at pH 8.0–8.4.

Our experiments are summarized in figure 2. Below pH 7.0 the fall in O_2 consumption is very slight and practically within the limit of error until about pH 6.5; thereafter it falls almost as a straight line function of pH to zero at pH 4. Between pH 6.5 and pH 8.7 a plateau of practically constant QO_2 occurs, similar to that seen in the case of liver, but over a smaller range. There is a hint of a slight optimum at about pH 8.5, but this is doubtful. Above pH 8.5 the rate of O_2 consumption diminishes sharply to zero at pH 10.

3. Testis. We are not aware of any previous observations of this nature on testis. The results we obtained will be seen in figure 3. The "plateau" of constant O_2 consumption is more sharply constricted than is that of liver or kidney, extending from about pH 6.7 to pH 8.3, with a

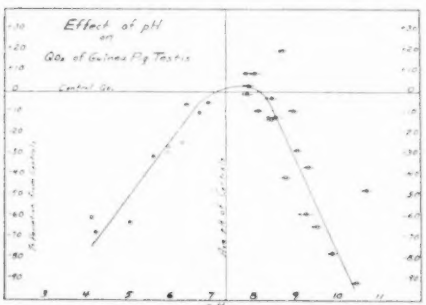

Fig. 3

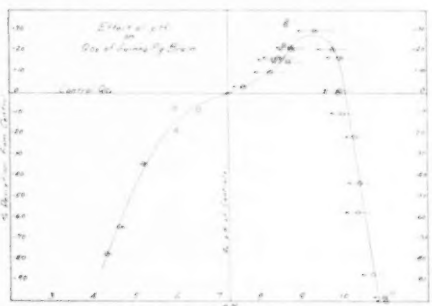

Fig. 4

slight hint of an optimum at pH 8, but again of doubtful significance. The slope of the curve indicates that zero is reached at about pH 3.5 on the acid side and pH 10.5 on the alkaline side.

4. Brain. Cohen and Gerard (1937) found that the respiration of a watery extract of rabbit brain showed very little difference in O_2 consumption between pH 7.2 and 8.0. So far as we are aware, these are the only relevant experiments on brain, apart from studies on individual enzyme systems. In the nerve fiber, Fenn and his collaborators (1934) found that in paired nerves the rate of O_2 consumption was 18 to 100 per cent higher at pH 7.6 than at pH 6.8. As will be seen in figure 4, the curve of O_2 consumption in relation to pH is different from that of the other tissues studied. Below pH 7.0 the QO_2 decreases with greater acidity much as in the case of the other tissues. On the alkaline side, however, the rate of O_2 uptake increases unmistakably to a fairly sharp optimum at pH 9.0

9.5, when the QO_2 is about 30 per cent elevated, then begins to fall abruptly, reaching zero at about pH 11.

Discussion. In changing the pH of the medium, we have directly added only Na and Cl. In the curve of brain metabolism (fig. 4) there are three points at pH 8.6 marked by a cross within a circle. In these experiments the serum, instead of being made bicarbonate free with acid and then brought to pH 8.6 with NaOH, was allowed to come to this pH simply by shaking to get rid of CO_2. Thus no Na was added. It will be seen that the three points were slightly higher than the rest of the curve, indicating a slightly depressing effect of excess sodium, not quantitatively significant. At pH 7.2 the concentration of Na in the serum was 0.14M; at pH 8.5 it was 0.155M; at pH 10 it was 0.17M. We have in unpublished experiments added NaCl to the medium in concentrations considerably greater than this without appreciable change in the QO_2. The effect of Na and Cl as well as osmotic changes can, we feel, be safely neglected in accounting for our results.

On the other hand, changing the pH influences the ionization of other substances, such as calcium and phosphate. Space does not permit our going into the literature on this subject. In regard to calcium, the evidence is somewhat conflicting, the conflict being in part perhaps due to concentrations and to the type of tissue studied. On the whole, excess calcium appears to depress metabolism. Phosphate, on the other hand, generally increases it. To what extent the effect of pH may in our experiments be a function of concomitant changes in calcium and phosphate ions, requires further investigation. In any case, however, the primary cause was the change in pH.

The most striking thing about the O_2 uptake is its behaviour beyond the ranges of the viability of the whole organism. Even on the acid side, though the QO_2 began to fall at once when the pH was on the acid side of neutrality, the O_2 uptake at pH 6.5 was still 80 to 95 per cent; at pH 6.0, 65 to 85 per cent; and at pH 5.0, 35 to 55 per cent of that at pH 7.3. On the alkaline side the O_2 uptake did not go below the level at physiological ranges in any case until well beyond the viability limits of the whole organism. In brain the optimum at pH 9.0 to 9.5 seems to indicate enzyme-substrate systems quite different from those of the other tissues. In the latter, the constancy of the QO_2 over a wide range would seem to imply either that the aerobic oxidations were not at all affected by changes in pH, or that as one enzyme-substrate system passed its optimum others took its place quantitatively, which seems improbable.

The fact that in all tissues studied the QO_2 was not depressed until well beyond the viability range—say from pH 6.8 to 8.0—raises interesting questions as to the reasons for the death of the organism beyond these limits. On the alkaline side death appears to be associated with phe-

nomena secondary to heightened irritability of the nervous system; on the acid side, however, with depression of nervous function. It may be: *1*. That the aerobic oxidations of certain master tissues, as for example the respiratory center, are peculiarly susceptible to changes in pH. Of this we have no evidence. *2*. That the vital functions depend on anaerobic oxidations, and that when a change in pH kills an organism, at least on the acid side, it does so by interfering with certain of these reactions. But the continuance of aerobic reactions would depend upon the continuance of the anaerobic oxidations, and although the former might go on for some time after cessation of function, they should fall off rapidly with time, which did not occur in our experiments. *3*. That neither aerobic nor anaerobic oxidations are disturbed appreciably at the time the organism has ceased functioning but that the application of released energy is interfered with, as for example at synaptic junctions. This seems to us the most plausible hypothesis.

SUMMARY

1. The effect was studied of changes in pH on the in vitro O_2 consumption of guinea-pig liver, kidney, testis and brain. In the first three the QO_2 was constant over a wide range, in brain there was a rise on the alkaline side, with a fairly sharp optimum at pH 9.0 to 9.5.

2. The QO_2 fell to zero at roughly the same levels of pH in all the tissues.

3. No appreciable fall in the QO_2 (as compared with that at pH 7.3) was observed in any tissue within ranges beyond the viability limits of the organism.

4. The relation of pH to viability is discussed.

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THE OXYGEN CONSUMPTION OF GUINEA-PIG TISSUES IN VARIOUS MEDIA[1]

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Received for publication June 1, 1939

In making observations on tissue respiration, we have had occasion to use different media, and have noted differences in the oxygen consumption of the tissues depending upon the medium. These differences have in some instances been so consistent and striking that we have analyzed the observations, and have conducted further experiments on individual tissues in more than one medium. A surprising difference has appeared in the behavior of brain as compared with other tissues of the guinea pig, as will be developed below.

METHODS. The oxygen consumption was studied by means of a Warburg-Barcroft differential manometer system. Most of the observations here summarized were made as controls for experiments instituted for other purposes; and are included in the table in the "average of all experiments." It is well known, however, that the QO_2 of tissues from different animals is apt to vary, sometimes rather widely, hence a purely statistical treatment of the effect of changing the medium has elements of danger. We have therefore conducted "paired" experiments, in which portions of the same tissue, after pooling and otherwise identical treatment, were placed in different media at physiological pH. The media used were 0.85 per cent NaCl and Ringer's solution with varying concentrations of a Sorensen phosphate buffer mixture, with and without glucose; and bicarbonate-free horse serum, with and without added phosphate buffer and glucose. The serum used was obtained from Massachusetts State Antitoxin Laboratories, and contained about 50 mgm. per cent reducing substances, but no glucose. For the most part, the experiments lasted one hour, and the observations included in the table are for this period of time, but we have on various occasions extended the experiments for a total time of not above two hours. Since the effect of time has not altered our conclusions, we have not included data on the metabolism after the first hour.

[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

TABLE 1

QO_2 of guinea-pig tissues in various media

	NaCl	NaCl-M/180 glucose			Ringer's	Ringer's-M/180 glucose		CO_2-free horse serum		
	M/75 PO_4	M/150 PO_4†	M/75 PO_4	M/30 PO_4	M/75 PO_4	M/150 PO_4	M/75 PO_4		M/150 PO_4 added	M/150 PO_4 + M/180 glucose added
Liver										
"Paired" experiments*		4.0±0.1						9.2±0.2		
		5.0±0.5						9.1±0.3		
		4.2±0.3						7.4±0.2		
		4.5±0.2						8.1±0.2		
			3.4±0.2					12.6±0.2		
		3.5±0.1						7.2±0.4		
		3.2±0.1						9.0±0.4		
	3.3±0.1		3.6±0.1						7.4±0.2	7.5±0.1
Average of all experiments		3.9 (101)‡	4.5 (3)	4.4 (16)				8.8 (75)		
Kidney										
"Paired" experiments*			13.6±0.7					23.8±0.8		
			13.1±0.5				11.6±0.3			
			16.0±0.1				13.3±0.1		20.0±0.9	
			16.5±0.3				14.2±0.8		19.7±0.3	
	13.5±0.4		19.7±0.1						23.5±0.4	23.8±0.8
Average of all experiments		14.4 (2)	15.9 (8)			14.5 (1)	15.3 (7)	18.8 (38)	21.1 (3)	
Testis										
"Paired" experiments*			9.6±0.5					9.3±0.1		
			9.7±0.7					9.0±0.3		
			9.2±0.3				8.3±0.3			
	9.7±0.3		10.0±0.3		8.2±0.4		9.3±0.5		9.6±0.2	9.1±0.3
Average of all experiments		9.2 (1)	9.5 (7)			8.7 (2)	8.7 (3)	10.1 (29)		
Heart										
"Paired" experiments*			6.5±0.5				5.7±0.4			6.6±0.4
	3.2±0.6		4.1±0.1							5.1±0.1
		2.7±0						4.1±0.2	3.8±0.2	
Average of all experiments		4.5 (4)	4.6 (5)					7.3 (7)		6.2 (3)
Brain										
"Paired" experiments*			11.8±0.8					7.6±0.1		
			16.0±0.1					9.2±0.3		
			17.3±0.3					9.5±0.7		
			13.6±0.8					9.3±0.4		
			11.0±1.1				11.8±0.4		8.5±0.1	
			12.3±0.1						9.4±1.0	
							11.5±0.2			8.7±0.4
			10.1±0.2				11.8±1.3			9.6±0.1
			11.6±0.7						7.2±0.8	8.4±0.6
			10.3±0.7						8.3±0	8.6±0.2
	2.9±0.6		7.5±0.2		2.5					6.8±0.2
	2.1±0.3		10.3±0.4		5.1		11.0±0.3		7.2±0.5	7.3±0.2
Average of all experiments	2.5 (2)		12.2 (12)		3.8 (2)		11.5 (4)	8.2 (29)	8.1 (5)	8.2 (6)

* By "paired" experiments we mean those in which the QO_2 of pooled slices from the same tissue was established in different media. Each experiment is on a separate line.

† Thirty experiments were performed on liver in NaCl-M/150 PO_4-M/180 glucose after incubation in horse serum exposed to 5 per cent CO_2/95 per cent O_2. The average QO_2 after 4 hours' incubation was 6.0 (25 experiments); after 10–12 hours' incubation the QO_2 was 5.5 (5 experiments).

‡ Numbers in parentheses are the number of experiments conducted.

In the "paired" experiments, the tissues were always sliced (or in the case of testis, teased apart) in 0.85 per cent NaCl-M/150 phosphate buffer medium, without glucose. The brain slices included both cortical and subcortical tissue. There was an interval of about one hour between the killing of the animal and the beginning of the experiment, including the time necessary for slicing or teasing, introduction into the flasks, and equilibration. The animals used were allowed food *ad lib.*

RESULTS. 1. *Liver.* The oxygen consumption was more than 100 per cent greater in serum than in the NaCl-PO_4 medium, as will be seen in the table. This discrepancy was not affected by changing the concentration of PO_4 in either the salt mixture or the serum. The addition of M/180 glucose to the salt and the serum in one experiment did not change the QO_2 in either medium, confirming findings of previous investigators.

2. *Kidney.* Dickens and Simer (1931) found the QO_2 of rat kidney to be practically the same in bicarbonate Ringer's solution, phosphate Ringer's solution, and horse serum. Dickens and Weil-Malherbe (1936) confirmed this for guinea pig kidney; and Weil-Malherbe (1936) found the O_2 uptake to be the same for both rat and guinea-pig kidney in NaCl-PO_4 as in dialyzed horse serum. On the other hand, Karczak (1933) found that the addition of ultrafiltrate of human serum, as well as cerebrospinal fluid, had a tremendous effect on the QO_2 of guinea-pig kidney in Ringer's solution. Our own findings were that O_2 consumption of kidney was practically the same in NaCl-PO_4 and Ringer's-PO_4, while in serum it was about 30 per cent higher. In all media, increasing the PO_4 concentration elevated the QO_2 slightly.

3. *Testis.* The only relevant previous experiments on testis seem to have been those of Dickens and Simer, who found no difference in the QO_2 as between Ringer's solution and serum. Our findings corroborate this, the O_2 uptake in NaCl-PO_4, Ringer's-PO_4 solution, and serum being practically identical, with or without added PO_4 or glucose.

4. *Heart.* The QO_2 in serum was low in all media. In one "paired" experiment there was practically no difference between the metabolism in NaCl-PO_4 and serum, but on the average the O_2 consumption in serum was 35 to 60 per cent higher.

5. *Brain.* Dickens and Simer (loc. cit.) observed no significant difference in the O_2 uptake of rat brain in Ringer's-bicarbonate solution, Ringer's-PO_4 solution, and horse serum. Quastel and Wheatley (1932), however, found that serum restored to normal the O_2 consumption of rabbit and mouse brain that had fallen during an experiment where no substrate was added. Our own results have been quite different. The average QO_2 in NaCl-glucose-PO_4 was 12.2; in Ringer's-glucose-PO_4 solution, 11.5. These experiments had to be carried out with M/75 PO_4 concen-

tration, as the buffering power of M/150 PO_4 was not sufficient to prevent a fall of pH of brain to unphysiological levels, which introduced an unwanted variable into the experiments. Brain was the only tissue we studied where this was necessary, for the fall in pH was rarely more than 0.3 pH in the other tissues, and in the case of the heart did not occur at all, in fact in several experiments actually rose. Compared with the results in NaCl and Ringer's solution, the QO_2 in serum was consistently depressed, averaging 8.1.

It has been consistently found by many observers that the addition of glucose to a Ringer's solution or NaCl medium elevates the O_2 consumption of brain. This was our experience as well, in fact the relative increase we observed was considerably greater than has been commonly found. Quastel and Wheatley found that the ability of added serum to elevate brain metabolism was similar to that of added glucose and lactate, and assumed that the serum effect was due simply to added substrate. It might be argued that since the serum we used contained no glucose, the depression of O_2 consumption in serum was due to the lack of substrate. However, glucose added to the serum in the same concentration as in the NaCl and Ringer's media had no observable effect on the metabolism in serum, hence presumably there was present in the serum adequate substrate for brain oxidations. As the QO_2 of brain in NaCl and Ringer's solution had to be carried out with M/75 PO_4, which is about double the normal concentration in serum, the PO_4 concentration in the serum medium was increased in some of the experiments, without demonstrable effect on the QO_2. Both lack of glucose and change in PO_4 concentration can therefore be ruled out as a cause of the observed depression of O_2 uptake in serum as compared with the other media. In respect to this effect of serum, brain is unique among the tissues studied, which suggests a fundamental difference in metabolism from that of the other tissues, a suggestion which is also implied in the response of the brain oxidations to changes in pH, reported elsewhere in this issue.

SUMMARY

1. The QO_2 of various guinea-pig tissues was observed in phosphate buffered NaCl and Ringer's solution and in bicarbonate-free horse serum, with and without added glucose, and with varying concentrations of PO_4.

2. The QO_2 of testis was about the same in all media. That of kidney, heart and liver was higher in serum than in either NaCl or Ringer's solution, the elevation being approximately 30, 35–60, and over 100 per cent respectively.

3. The QO_2 of brain, on the other hand, was about 50 per cent higher in the salt media than in serum, which was not to be accounted for by lack of substrate in the latter. This and other considerations suggest a quali-

tative distinction between the oxidative system of brain and that of other, non-nervous tissues.

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ELECTROPHYSIOLOGICAL STUDIES ON THE MOTILITY OF THE GASTROINTESTINAL TRACT

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Received for publication June 2, 1939

The action potentials during the normal movements of the stomach and small intestine will be described in this paper. In contrast to the great diversity of motility, the action potentials of these organs were found to be remarkably simple; they consist of bursts of brief spike potentials which vary only in duration, frequency and amplitude. In the light of previous experiments on isolated muscle (4, 5) these electric changes must be interpreted as impulses conducted within the syncytial muscle tissue. Each movement usually is accompanied by a repetitive discharge and is, therefore, analogous to a tetanic contraction of skeletal muscle.

Studies on isolated preparations led to the conclusion that visceral muscles are syncytia like cardiac muscle (4, 5). This result does not disagree with the well known fact that contractions of the viscera may remain fairly localized. It has been shown that conduction occurs only if the level of excitability is sufficiently high and that various factors like previous activity, hormones and nerve impulses (4, 5 and unpublished) produce wide fluctuations of excitability.

From the results on isolated visceral muscle, which are confirmed by the studies reported in this paper, it may be concluded that grading of the strength of contractions is largely due to variations in the number and frequency of impulses discharged during each contraction. In addition, partial contractions of muscles occur frequently if the excitability of the muscle is low (p. 304).

The action potentials of the intestine had been investigated previously (cf. 3, 6, 8), but attention was devoted chiefly to slow potential changes which followed fairly closely the movements of the organ. It will appear from the following that these slow changes are a phenomenon entirely different from the spike potentials described in this paper. Slow potential changes were frequently recorded also in my own experiments, but their magnitude was greatly diminished by a suitable arrangement of the leads and they could often be avoided entirely. It was concluded, therefore, that the slow changes were artifacts due to movements of the muscle.

It has been suggested that the action potentials led off from visceral

organs might arise from the enclosed nervous plexus (3). However it seems inconceivable that the potentials of these nervous elements could be detected because the plexus is imbedded in a large mass of tissue and because studies on nerve trunks have shown that thin nerve fibers, as they occur in the viscera, give only weak action potentials. Additional evidence for the muscular origin of the action potentials of visceral muscles may be found in previous papers (4, 5).

METHODS. Exposed loops of intestine of anesthetized animals were studied in the majority of experiments. As anesthetic, nembutal was used for cats and dogs; urethane, supplemented by intravenous injections of nembutal, was used for rabbits. The depth of the anesthesia had no influence on the results. Cats and dogs were deprived of food for two to three days before the experiment because the intestinal movements then were more vigorous.

A loop of intestine was exposed and the animal, with the exception of the head, was enclosed in an insulated and shielded box which was electrically heated and carefully humidified. A glass window permitted visual observation of the intestinal movements. Warm Ringer's solution was dripped on the exposed loop from time to time.

In the guinea pig it was found convenient to record action potentials from isolated segments (6 cm. long) of the small intestine. After removal from the animal the preparations were immersed in Ringer's solution and then mounted in the muscle chamber previously described (5). One end was tied off, the other end was attached to a glass tube which permitted the inflation of the organ from the outside. The conditions were similar to those in Trendelenburg's experiments (9) except that the intestine was kept in air instead of Ringer's solution.

The action potentials were recorded by an ink writer activated by a condenser coupled amplifier. The time constant was 0.2 second, except in the experiments on guinea pigs where it was 2 seconds. If the time constant was short, slow potential changes as they frequently arose from movements were largely eliminated, whereas the spike potentials were not noticeably distorted.

Artifacts produced by movements probably arise from the unequal concentration of electrolytes on the surface of the organ due to evaporation or condensation of water. The difficulty of preventing some drying of the exposed intestine in a large container explains why the artifacts were much larger in this type of experiment than in experiments on isolated muscles permitting the use of a small muscle chamber. Thin cotton wicks soaked in Ringer's solution and flexible enough to follow the movements of the intestine were found to give smaller movement artifacts than any of the other arrangements tested. In many experiments the intestinal loops were supported on a glass rod so as to diminish movements.

It would be desirable to record action potentials by a monophasic lead, but this is impractical because injury must be avoided in a study of normal intestinal movements. The two leads were placed on the intact organ at a distance of 5 mm. or less, thereby recording the electric changes from a small region of the muscle. If the distance between the leads was greater, the action potentials were more complicated, as might be expected from the fact that the recorded potentials are influenced by the whole mass of active tissue between the leads. This difficulty, in connection with the movement artifacts, explains why previous investigators failed to obtain the simple results which will be described in this paper.

Results. In the intestine of the guinea pig three distinct types of activity were observed. The isolated segments, particularly if slightly inflated, often showed pendular movements which were indistinguishable from those of the organ in situ and had their characteristic rapid frequency of one beat every 1.2 seconds. Each beat was accompanied by a discharge of one to three impulses (fig. 1A and B).

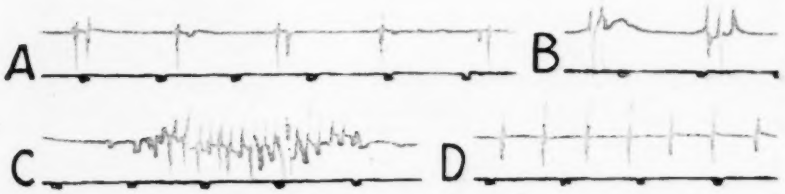

Fig. 1. Action potentials from isolated segments of the small intestine of the guinea pig. A and B, pendular movements; C, peristaltic contraction elicited by distention; D, slow discharge. Time marks every second. Temp. 37°.

Inflation elicits, in the quiescent organ, peristaltic contractions as described by Trendelenburg (9) and others (cf. 7). They are conducted over the whole muscle at a rate of one to two centimeters per second and are accompanied by a discharge lasting for about three seconds (fig. 1C). Every inflation elicits a series of these bursts of impulses.

After strong inflation the peristaltic movements are sometimes followed by a slow and very regular discharge lasting for several minutes at a gradually diminishing frequency (fig. 1D). At low frequencies of discharge one can observe waves of contraction, appearing like minute ripples, passing along the intestine, each one corresponding to a single impulse. This long continued discharge probably corresponds to the slow contractions observed by Henderson (7) under similar conditions.

Each pendular movement of the rabbit's small intestine is accompanied by a discharge of 3 to 15 impulses. The duration of this discharge and the

height of the impulses increases with the amplitude of the contractions (fig. 2).

A peristaltic rush of the small intestine of the rabbit is accompanied by a burst of strong impulses discharged at a frequency of 7 to 10 impulses per second and lasting usually for about 10 seconds (fig. 4A). This type of activity was elicited by introducing a bolus of cotton. The movement generally started after a considerable amount of intestinal secretion had accumulated on the oral side of the bolus. Rhythmic contractions were absent in the strongly distended region of the intestine. The rush always originated at a considerable distance from the bolus and on its oral side. If the contraction failed to move the bolus the process was repeated after a period of rest lasting for at least a minute.

The empty small intestine of the cat often showed segmenting movements, circular constrictions extending over a centimeter or less. During these contractions one or two, rarely three strong impulses were discharged

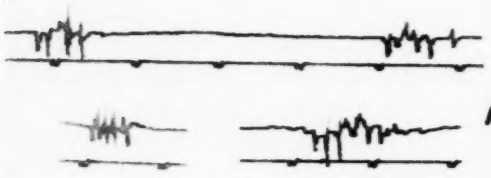

Fig. 2

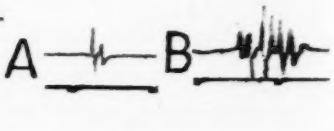

Fig. 3

Fig. 2. Action potentials during the pendular movements of the small intestine of the rabbit.

Fig. 3. Action potentials from the small intestine of the cat. A, segmenting movement. B, wave of contraction.

(fig. 3A). In some animals waves of contraction traveled along the intestine over a distance of 10 to 20 cm. at a rate of a few centimeters per second. They were accompanied by a discharge lasting for about a second (fig. 3B). Often this kind of activity gradually changed over into the more usual segmenting movements. The fact that during segmenting movements fewer impulses were discharged and that longitudinal conduction was restricted to a region of a centimeter or less suggests that this type of contraction occurs if muscular excitability is lower than during the appearance of waves of contraction.

The motility of the cat's intestine was greatly increased by moderate distention. A bolus of cotton wool lubricated by vaseline was introduced into the small intestine through a longitudinal incision. Strong rhythmic contractions on one or both sides of the bolus were produced. In a few cases strong movements of this type on the oral side of the bolus transported the bolus slowly aborally. These movements were essentially like the

peristaltic movements which Bayliss and Starling (1, 2) described for the intestine of the dog, but which they had been unable to obtain in the cat.

In the dog peristalsis of the small intestine can be induced more readily than in the cat. This type of activity progresses along the intestine very slowly (usually 1 to 2 cm. a minute, often less). The movements consist of rhythmic waves of contraction which originate in the rear of the bolus, travel rapidly towards it and fade out in the distended region of the intestine. Each one of these waves pushes the bolus forward by about a millimeter. Antiperistalsis was observed several times but it moved the bolus for only a short distance.

Leads were placed so that the bolus had to pass underneath. Each wave of contraction was found to consist of a burst of impulses. The strength

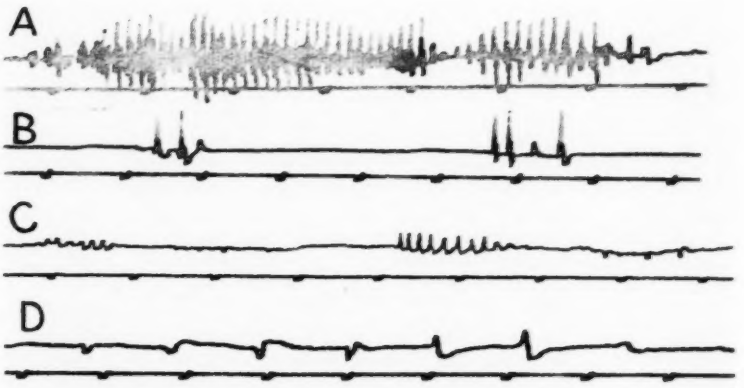

Fig. 4. Action potentials during peristalsis. A, peristaltic rush in the small intestine of the rabbit. B, rhythmic peristalsis in the small intestine of the cat. C, the same in the dog. D, single peristaltic wave of the empty stomach of the cat.

of the impulses was strongest just in the rear of the bolus. The potentials recorded often were monophasic indicating that they often stopped between the leads (fig. 4B, C).

The peristaltic movements in the small intestine of the cat and dog are evidently quite different from the peristaltic rush as observed in the rabbit. The former consist of rhythmic contractions each of which is conducted only over a distance of two to three centimeters. The progression of the whole wave evidently depends in this case largely on the mechanical conditions and is much slower than the velocity of conduction of impulses. The peristaltic rush, on the contrary, is a single rapid wave of contraction. The question why the peristaltic contractions usually arise on the oral side of a bolus, showing the existence of some polarity in the organization of the intestine, is outside the scope of this paper.

Action potentials were led off from the cat's stomach. During each peristaltic wave 5 to 10 impulses were discharged at a frequency lower than that observed in any other type of visceral muscle (fig. 4D).

It is significant that the magnitude of the spike potentials during the various types of activity of the intestine varies within wide limits. During weak pendular movements the potentials were often less than 20 microvolts, whereas during peristalsis they reached values of nearly 1 millivolt in the rabbit and 4 millivolts in the cat and dog. It is true that the recorded curves give only a rough indication of the potential changes because of the use of a diphasic lead. However, the large variations in the size of the potentials are correlated with variations in the strength of the contractions and evidently represent real differences. That movements which are accompanied by strong action potentials, like intestinal peristalsis, are due to much stronger contractions than pendular movements is shown by the fact that the organ blanches during the former but does not change its color during the latter type of activity.

These observations are not contrary to the all or none relation which has been demonstrated in experiments on isolated visceral muscles (4). Variations in the size of the action potentials may have two causes. The magnitude of the response may change under different conditions as found also in other types of muscles. Direct observation, furthermore, indicates that during weak contractions only small regions of the muscle contract actively. It is reasonable to assume that a condition of low excitability limits conduction of the impulses during such contractions.

SUMMARY

The action potentials of the stomach and small intestine were recorded during the normal muscular activity, pendular and segmenting movements and peristalsis.

The movements of these organs are accompanied by a discharge of brief action potentials. In the light of previous work on isolated visceral muscle these potentials must be interpreted as impulses conducted within the syncytial musculature.

Usually each contraction is accompanied by a repetitive discharge and is, therefore, analogous to a tetanic contraction of skeletal muscle. The frequency of discharge ranged from about 1 per second during peristalsis of the stomach to 10 per second during a peristaltic rush in the small intestine of the rabbit.

Grading of the contractions is possible by a variation in the number and frequency of impulses. In addition the size of the impulses varies with the strength of the contraction, probably because only part of the musculature is active during weak contractions.

The peristalsis in the small intestine of the cat and dog consists of

rhythmic contractions on the oral side of a bolus, whereas the peristaltic rush in the rabbit is a continuous wave of contraction.

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THE INFLUENCE OF THE PANCREAS AND THE LIVER ON THE OXIDATION OF ETHYL ALCOHOL[1]

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Received for publication May 5, 1939

The oxidation of ethyl alcohol may be depressed by inanition (1) and accelerated after the administration of various foodstuffs (2). That insulin may play a part in this phenomenon has been suggested by the studies of several investigators (3, 4, 5). More recently, Clark and Morrissey (6) reported that the administration of glucose and insulin increased the rate of alcohol removal from the blood of normal dogs and therefore postulated that the oxidation of ethyl alcohol may be dependent upon the simultaneous oxidation of glucose. In a subsequent report, Clark, Morrissey and Fazekas (7) demonstrated that the liver brei from the non-insulinized depancreatized cat cannot oxidize alcohol to any degree comparable with that from the normal cat, from which they concluded that insulin is necessary for the oxidation of alcohol.

The resemblance between this concept and the hypothesis that the oxidation of acetone bodies is dependent upon the simultaneous oxidation of glucose, led Goldfarb and Bowman (8) to perform a series of "in vitro" experiments similar to those of Shaffer's (9). They demonstrated that the oxidation of ethyl alcohol by hydrogen peroxide in an alkaline solution was accelerated when glucose was simultaneously undergoing oxidation. This observation is identical with that of Shaffer for acetoacetic acid and lends support to the hypothesis of Clark and Morrissey.

In view of the fact that in spite of "in vitro" evidence to the contrary, the "in vivo" utilization of acetone bodies is independent of that of carbohydrate (10), it became of interest to us to find whether or not further "in vivo" studies would reveal the existence of an interrelationship between carbohydrate and alcohol oxidation. Towards this end, we investigated the influence of the pancreas and the liver on the utilization of ethyl alcohol.

METHODS. The rate of alcohol utilization was computed from the rate at which alcohol disappeared from the blood stream of unanesthetized

[1] Presented before the American Physiological Society, Toronto, April 28, 1939.

[2] Eli Lilly Fellow.

animals after the intravenous injection of one gram of ethyl alcohol (in 20 per cent solution) per kilogram of body weight. In all instances, at least twenty minutes were permitted to elapse between the injection and the time at which the first blood sample was drawn from the external saphenous or external jugular vein. Samples were drawn at various intervals thereafter, and their alcohol and sugar contents determined by the methods of Friedemann and Klaas (11) and Somogyi-Shaffer-Hartman (12) respectively.

The first set of experiments consisted in establishing the rate of alcohol utilization by normal dogs after a preliminary fast of from 18 to 24 hours and of increasing periods thereafter up to 144 hours. In a second series of dogs the influence of pancreatectomy with and without the administration of insulin and glucose was studied. In every instance control alcohol curves were obtained on two occasions before pancreatectomy. The third series of experiments was performed with rabbits which were permitted to eat until a few minutes before the experiment, when they were nephrectomized and then allowed to recover from anesthesia. They were then used to establish the normal rate of alcohol utilization. Another group of rabbits was eviscerated and after allowing for complete recovery from the anesthesia, their rate of alcohol utilization was likewise determined, the blood sugar being maintained by the intravenous injection of glucose. Some of these eviscerated rabbits were maintained at normal blood sugar levels while others were maintained at levels of from 500 to 700 mgm. per cent. To a group of eviscerated rabbits with both normal and high blood sugar levels, from 1 to 2 units of insulin per kilogram of body weight per hour were administered intravenously. Blood samples for alcohol and sugar determinations were drawn from an exposed femoral artery.

The fourth set of experiments was performed with rabbits after partial hepatectomy and alcohol curves subsequently obtained. The procedure in these experiments consisted in removing the kidneys and one or more lobes of the liver under ether anesthesia, closing the abdominal wound and permitting the animal to recover from the anesthesia. Ethyl alcohol was then injected and after a twenty-minute interval a blood sample was drawn, and again at two and four hours. The animal was then sacrificed and the liver removed. By weighing the portion of liver removed at operation and that remaining in the abdomen, the per cent removed was computed.

The fifth series of experiments was performed with dogs after exposing them to chloroform anesthesia for various intervals of time.

Results. In order to conserve space, typical experiments are illustrated in figures 1 to 6.

The rate of the removal of alcohol from the blood of normal dogs is

depicted in figure 1. This is observed to be fairly uniform from period to period. However, individual animals were found to vary occasionally from one another, thus making it necessary to establish the rate for each animal.

The influence of fasting did not become apparent in our animals until after 96 hours had elapsed, at which time a small decrease in the rate of alcohol removal from the blood stream was observed (fig. 2). The administration of food to such an animal results in a complete restitution to the normal rate.

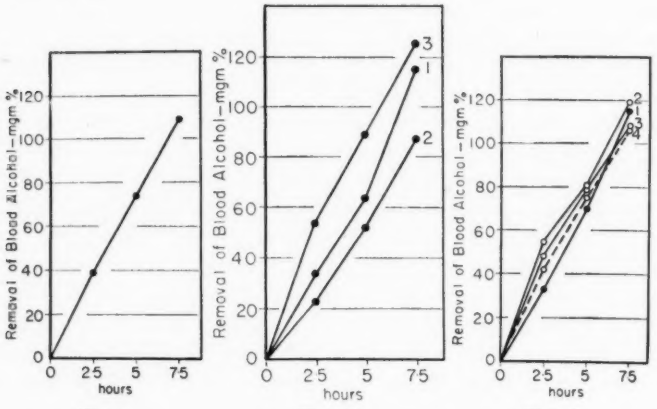

Fig. 1 Fig. 2 Fig. 3

Fig. 1. The average of 18 control alcohol curves in normal dogs after a preliminary fast of from 18 to 24 hours.

Fig. 2. Illustrating the influence of fasting on the rate of alcohol removal from the blood. *1* and *2* refer to curves obtained after 24 and 96 hours' fasting respectively. *3* was obtained 24 hours later, during which interim the animal was fed.

Fig. 3. Illustrating the influence of pancreatectomy on the removal of alcohol from the blood stream. *1*, before pancreatectomy; *2* and *3*, 64 and 88 hours after pancreatectomy respectively; *4*, 48 hours later, after treatment with insulin and glucose.

The influence of pancreatectomy is illustrated by the typical experiment in figure 3. Only one control curve is depicted although two such curves were always obtained. It may be noted that curves 2 and 3, obtained 64 hours and 88 hours respectively after pancreatectomy, differ very little from the control. For the next 48 hours this animal was treated with insulin and glucose and then curve 4 was obtained, which again varied little from the preceding curves. In other depancreatized dogs we have occasionally observed a depression in the rate of alcohol removal about 96 hours after pancreatectomy, but when this did occur the magnitude was of the same degree as that observed in normal dogs which have been fasted

96 to 144 hours. These results suggest that insulin is not essential to the oxidation of alcohol and that when a decrease in alcohol utilization does occur after removal of the pancreas, it is due to some factor other than the lack of insulin per se.

In figure 4 are depicted some of the data obtained with nephrectomized and eviscerated rabbits. Curve *1* illustrates the rate of alcohol utilization by nephrectomized rabbits at various blood alcohol levels. It is obvious from this that the rate is much more rapid in the rabbit than it is in the dog. When the viscera are removed and the blood sugar maintained at a normal level (75 to 123 mgm. per cent) no apparent removal of alcohol from the blood is observed (curve *2*). Maintaining the blood sugar at

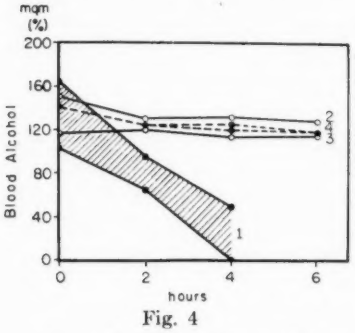

Fig. 4

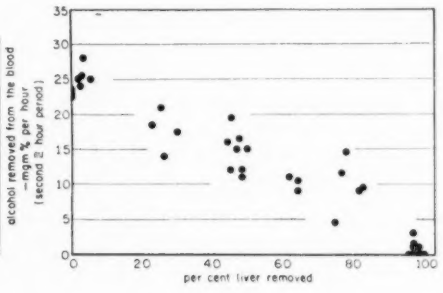

Fig. 5

Fig. 4. Illustrating the utilization of alcohol by nephrectomized and eviscerated rabbits. *1*, nephrectomized rabbits; *2*, eviscerated rabbits with normal blood sugar level; *3*, eviscerated rabbits with high blood sugar level; *4*, eviscerated rabbits with high and low blood sugar levels receiving insulin at hourly intervals.

Fig. 5. Illustrating the influence of partial hepatectomy on the rate of alcohol removal from the blood of rabbits during the last two hours of a four hour experiment. Upon dividing up the abscissae and ordinates into groups of 10, statistical analysis reveals that $r = -0.947$, $\sigma_r = 0.008$, $b_{xy} = 1.05$, $b_{yx} = 0.829$.

high levels (500 to 700 mgm. per cent) produced no appreciable influence on alcohol utilization (curve *3*). The administration of insulin was likewise ineffective in either group of rabbits (curve *4*). It may be noted that in many instances, a small amount of alcohol is removed from the blood of eviscerated rabbits during the first two hour interval but that subsequently the disappearance of alcohol is not appreciable, which definitely indicates that the muscles do not utilize ethyl alcohol. The fact that insignificant changes occur after the two hour interval is in accord with the work of others who demonstrated that relatively insignificant amounts of alcohol are lost via the lungs.

Because of our findings with the completely eviscerated rabbit, it became of interest to investigate the influence of partial and complete hepatec-

tomies. Figure 5 depicts the findings in a series of rabbits from which various amounts of liver were removed before obtaining the alcohol curve. Only the second two hour utilization is plotted in this figure because of the fact mentioned above, that during the first two hours some diffusion may still be going on. However, it must be emphasized that the utilization per hour for the total experimental period of four hours varied little from that during the last two hours. These data reveal a striking relationship between the rate of alcohol removed from the blood and the per cent of liver present. Thus, on statistical analysis we find that the coefficient of correlation (r) between the alcohol removed from the blood per hour and the per cent of liver removed from the animal at the beginning of the experiment is -0.947. The standard error of this correlation (σ_r) is 0.008. Furthermore, the calculation of the regression coefficient reveals that

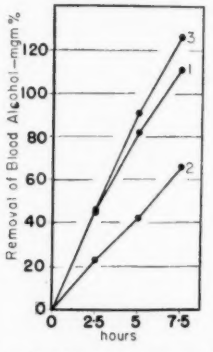

Fig. 6. Illustrating the effect of chloroform anesthesia on alcohol utilization. *1*, control; *2*, after being subjected to chloroform anesthesia for 15 minutes per day for 5 days; *3*, 12 days after cessation of chloroform anesthesia.

$b_{xy} = 1.05$ and $b_{yx} = 0.829$. Finally, calculation of the correlation ratio indicates that the distribution of the data is rectilinear.

In view of the preceding it became of interest to study the effect of some hepatoxin on the rate of alcohol utilization. Chloroform anesthesia in dogs was used for this purpose and a typical example is depicted in figure 6. Curve *1* was obtained before anesthesia was induced. Curve *2* was obtained after the animal had been exposed to chloroform anesthesia for 15 minutes per day for 5 days and curve *3*, 12 days after the cessation of chloroform anesthesia. It is obvious from such experiments that chloroform anesthesia produces a definite depression of the rate at which alcohol is removed from the blood, which is in accord with the data that even a partial hepatectomy will decrease the utilization of alcohol.

Discussion. The present study does not provide evidence in support

of the concept that insulin is essential for the oxidation of ethyl alcohol, or that glucose exerts a catalytic effect in that regard, since we have found that the acutely depancreatized dog shows little or no impairment in its ability to use alcohol. Furthermore, the fact that the eviscerated rabbit does not utilize alcohol and that neither the administration of large quantities of glucose nor insulin is effective in this regard, suggests that the "in vitro" experiments of Goldfarb and Bowman are not of physiological significance.

In a recent report, Lundsgaard (13) presented data obtained from artificially perfused isolated livers and hind-limb preparations which led him to conclude that alcohol is rapidly oxidized in the liver, but is not effectively oxidized at all in a hind-limb preparation. Our experiments with unanesthetized abdominally eviscerated rabbits are in complete accord with Lundsgaard's observations and suggest that alcohol oxidation is a specific function of the liver. This possibility receives strong support from the observation that a quantitative relationship can be demonstrated between the per cent of liver remaining intact in a partially hepatectomized animal and its ability to remove alcohol from the blood. In this respect alcohol utilization differs from other functions of the liver which usually show no significant impairment until over 80 per cent of the liver is removed. The fact that chloroform anesthesia, which is known to produce hepatic damage, can depress the rate of alcohol utilization, is in accord with the concept that we are dealing with a specific function of the liver. It is possible that a depletion of glycogen or an infiltration of fat into the liver may produce some change in hepatic function which expresses itself by a decrease in the rate of alcohol utilization such as is observed in fasted dogs.

The application of these observations to clinical material has precedence in the observation of Erwteman and Heeres (14) who found that the ingestion of alcohol by patients suffering from various types of hepatic disease revealed a disturbance in the "blood alcohol curve." These observations are subject to criticism because of the fact that variable rates of absorption may occur in various disease states. However, after intravenous administration, we have obtained data which suggest that further application of the above findings may prove significant.

SUMMARY AND CONCLUSIONS

1. The rate of alcohol utilization is depressed by fasting.

2. The acutely depancreatized dog shows no significant impairment of alcohol utilization and hence insulin is apparently not essential.

3. The unanesthetized eviscerated rabbit does not utilize alcohol irrespective of whether large amounts of glucose or insulin are administered, indicating that the muscles do not utilize alcohol.

4. A quantitative relationship is demonstrated between the per cent of intact liver of a rabbit, and its ability to utilize alcohol.

5. Chloroform anesthesia depresses the rate at which alcohol is removed from the blood.

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We are indebted to the Eli Lilly Co. for generous supplies of insulin.

STUDIES ON THE GENESIS OF ISCHEMIC PAIN: THE INFLUENCE OF THE POTASSIUM, LACTATE AND AMMONIUM IONS

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Received for publication June 14, 1939

Ischemic pain is the modality evoked by exercise of the properly weighted, non-circulated muscle. It has been described as a continuous but fluctuating deep-dull sensation, poorly localized within the belly of the muscle worked. Once evoked by ischemic work it characteristically persists without decrement, so long as the blood supply is occluded, though no further work is done. Once the blood supply is restored, it disappears in a few seconds.

The clinical importance of this modality lies in the fact that it is typical of the discomfort in intermittent claudication, as demonstrated by Lewis, Pickering and Rothschild (5). Angina pectoris of cardiac origin is thought to have an identical mechanism of genesis though proof of this hypothesis is lacking.

The pain in ischemic work is thought to be produced by the action of some catabolite of muscular activity on nerve endings between the muscle fibers. This catabolite was termed factor P (5). It is accepted to be a product of both aerobic and anaerobic contraction but is supposed to accumulate to a stimulating concentration only when anaerobic mechanisms predominate. It is probably diffusible and nonvolatile (3), and may be oxidizable. Evidence has been presented which suggests that intercellular acidity is not the stimulating factor (7a). It is not known which nerve fibers carry the modality, as the sensitive endings have not been located microscopically.

Elicitation of the modality by means other than work has been reported by Lewis (6) and Kellgren (4). They found that hypertonic saline solution injected into the muscle belly produced a pain identical in quality with that caused by ischemic work.

The present study was undertaken to determine the liminal painful concentration of some of the known products of muscular contraction, administered intramuscularly. Theoretically factor P should show certain characteristics upon intramuscular administration. First, the liminal

painful concentration should be less than isotonic as it is hardly conceivable that any one ion or substance should reach isotonic concentration alone. Second, the liminal concentration should be highest in the circulated muscle, least in the ischemic muscle worked only to an extent not capable of producing pain. This liminal concentration in the worked condition should be less than the maximum concentration developed by physiological activity. If then some single substance or ion could be found which would fulfill these criteria, identity with factor P would be suggested.

METHOD. The experiment is carried on in 2 or 3 stages. In the first part the liminal painful concentration of the ion in the normal muscle was determined. The subject was seated in a chair, with his arm (usually but not invariably the left arm) upon an arm rest. The extensor digitorum communis was outlined on the skin. The skin was cleansed with iodine and alcohol. Three intradermal wheals of 2 per cent procaine hydrochloride were made about 3 cm. apart over the belly of the muscle. Three twenty gauge 1½-inch needles were then inserted through the skin, the superficial and the deep fasciae into the muscle belly. Accurate placement of the needles is rendered easy by the twinge occurring when the needle goes through the deep fascia into the muscle.

If the arm, following insertion of the needles, is not painful, a preliminary injection of 0.3 cc. of 0.9 per cent NaCl solution is made through one of the needles. Later an injection of 3 per cent NaCl solution is made through another. These 2 injections demonstrate: 1, that it is possible to make these injections into this subject without producing pain; 2, what the distribution of pain is in this particular subject. No clue is ever given the subject as to what to expect from any injection.

A sterile isotonic solution of the substance to be tested and another of sodium chloride were then mixed in varying proportions in tuberculin syringes. No attempt has been made to determine limina closer than one-tenth of the concentration of the test solution. For example, the experiment determined that a mixture of 40 per cent of the test solution and 60 per cent of the NaCl solution is painful, whereas 30 per cent of the test solution with 70 per cent of the NaCl solution is not painful. In a random order then the various dilutions were tried. In so far as possible a limen was determined for each of the needles. About 12 injections were the maximum that could be done at one occasion.

At a later date, the second stage, the procedure was repeated, with the variation that a sphygmomanometer cuff was inflated about the arm to a pressure of 220 mm. Hg before each injection. With certain of the test solutions this stage could be combined with the first. The limen for the ischemic arm was thus determined.

After an interval of another week the last stage was done. The subject's forearm was placed upon the arm rest of an extensor ergograph and the extensor of the digits weighted with 600 grams. The forearm was then

rendered ischemic, as described above, and the muscle worked at the rate of 1 contraction per second until pain resulted. The number of contractions required was noted. The ischemia was then relieved. Ten minutes' rest was allowed before any further work.

Through a wheal of procain an intramuscular needle was then inserted into the superficial fascia. The forearm was again rendered ischemic, and the muscle worked one-half as many contractions as led to pain before. If no pain occurred, the needle was plunged through the deep fascia into the muscle belly and a solution injected. If no pain resulted in 1 minute, the ischemia was relieved. The needle was then pulled out of the muscle. Ten minutes were again allowed before another trial was attempted. Usually but two trials of work and injection through a single needle were made, and about 4 needles were used per experiment. Thus the limen for the arm worked to a pre-pain level was determined.

Finally in the same subjects after another interval the question of qualitative identity of injection pain and work pain was tested as follows: Two blood pressure cuffs were connected to the same pressure reservoir through a valve. One was applied to each arm of the seated subject. The right forearm was placed in the extensor ergograph, the left was prepared for injection through a single intramuscular needle. The arms were rendered ischemic simultaneously by opening the valve and the right muscle was worked until work pain was definite. Three per cent NaCl solution was then injected into the left extensor of the digits. A careful explanation was made that neither quantitative nor time relation similarity was to be judged, that only a judgment of difference in quality was desired. None of the subjects except the author were familiar with the implications of a judgment of similarity or dissimilarity. After the judgment had been given the ischemic state was relieved.

The test solutions were made by gravimetric methods. Analytical reagent potassium chloride was used in a concentration of 1145 mgm. per cent in distilled water which, if completely ionized, would contain 600 mgm. per cent of K ion. Analytical reagent ammonium chloride solution 850 mgm. per cent in distilled water should contain 280 mgm. per cent NH_4 ion. Free sarcolactic acid solution containing 1800 mgm. per cent in distilled water was neutralized to pH 7 with NaOH. All of these solutions were autoclaved in clamped serum bottles at 20 pounds for 30 minutes. After autoclaving, a sample was aseptically withdrawn and dried in a desiccator and the per cent of the solid of the solutions thus checked.

Results. A total of 39 subjects was used. Of these, 9 subjects were unsatisfactory because they reported pain on receiving 0.3 cc. of 0.9 per cent NaCl intramuscularly. In 2 of these 9 it was shown that the volume of the solution made no difference, pain being reported with 0.1 cc. just as with 0.3 cc. Three per cent NaCl solution was found to produce

pain uniformly in all but 1 of the 39 subjects. The pain produced by 3 per cent NaCl solution was described as rising slowly after the injection, reaching a peak in about 1 minute, and gradually diminishing over a period of as much as 4 minutes. The pain was called dull, deep seated, and said to waver somewhat in its intensity.

Site of pain. As to the location of the pain on injection into the extensor digitorum communis, 18 subjects had pain poorly localized to the muscle belly itself. Twenty had pain both in the muscle belly and over the dorsal surface of carpus, metacarpus or phalanges. One subject reported pain only over the carpus and metacarpus, without sensation in the muscle. Pain from ischemic work uniformly was in the muscle itself, more diffuse, and stronger than the injection pain. Only one subject reported reference of ischemic work pain to the dorsum of the metacarpus. Location of the pain seemed characteristic of the subject, not characteristic of the painfulness of the solution used.

K limen. The limen for the normal muscle was determined in most subjects for each of the three needles used. The results in 9 of the subjects were never more than one unit (60 mgm.) apart in the three needles. The average of the three limina was taken as the limen for the individual. The 13 individual averages thus obtained were used to derive the average limen of 225 mgm. per cent with a range from 180 to 300 mgm. per cent.

In the unworked ischemic arm only 5 subjects were tested. The limina so determined were identical or slightly higher than those obtained in the same arm in the normal state. There appeared to be less variability between the limina for the 3 needles, perhaps because the subjects had become accustomed to the procedure. The average limen was 210 mgm. per cent with a range from 180 to 270 mgm. per cent.

After the ischemic muscle had been worked to a pre-pain state the limina were determined in 10 individuals, and the average obtained was 220 mgm. per cent (minimum 180 mgm. per cent, maximum 270 mgm. per cent). In most of the cases the pain disappeared before the ischemia was relieved.

Sarcolactate limen. The solution used contained approximately 1800 mgm. per cent of lactate ion (assuming that the salt was completely ionized) at pH 7.0. The full strength solution was not painful in any of 10 subjects, whatever the condition of the muscle.

In 3 other subjects a lactic acid solution of similar lactate strength but neutralized only to pH 4.8 was used. Two of the subjects had very mild pain at 1800 mgm. per cent. One showed a limen of 1300 mgm. per cent.

In one subject a straight 1 per cent lactic acid solution was used (pH about 2.0). This produced very sharp pain immediately, and soreness for 72 hours following the injection. This soreness was subjectively identical with that resulting from excessive exercise of unacclimated muscles.

NH_4 limen. The 0.85 per cent NH_4Cl solution contained about 280 mgm. per cent NH_4 ion if completely ionized. The unit was thus 28 mgm. per cent. The average limen in the normal arm of 6 subjects was 213 mgm. per cent. One subject reported mild pain from 168 mgm. per cent; another only occasionally experienced pain from the full 280 mgm. per cent.

In the ischemic arm worked to a pre-pain level the average limen of 6 subjects was 214 mgm. per cent with a range from 168 to 280 mgm. per cent.

Acetyl choline. In one subject 300γ of acetyl choline in 0.3 cc. isotonic NaCl was tried. No pain resulted in the normal, in the ischemic, or in the worked muscle.

Identity of work and injection pains? Thirty subjects were tested as to the qualitative identity of work and injection pains experienced simultaneously. Twenty subjects found the quality exactly the same, agreed that stripped of the accompanying circumstances the origin of the pain could not be determined. Ten subjects stated that the quality of the pains was different. Five of these spontaneously remarked that the injection pain had a sharp quality whereas the work pain was dull. Four spontaneously stated that the injection pain was dull, the work pain sharp. In four of these ten the differences described were predominantly quantitative, and it was hard to be certain that the subject really perceived a qualitative distinction.

Discussion. The extensor digitorum communis muscle was used because it is superficial and thus readily accessible, because it lends itself well to the ergograph, and because certain data are available on the pH changes which occur in this muscle after the same type of exercise (7a).

An injection volume of 0.3 cc. was chosen empirically because it was feared that too small a volume might be too rapidly diluted or dispersed by diffusion in the intercellular fluid, and that too large a volume might be painful due to distention.

It is interesting to note that nearly 25 per cent of the subjects experienced pain from the injection of 0.9 per cent NaCl. In two subjects it was shown that the volume of the injection was not the causative factor. The others were not tested for this distention sensitivity. It is of course possible that the effect was a psychic one in some of the subjects, but attempts to demonstrate this by bringing the syringe to the needle and removing it without actual injection were not successful in producing pain.

The persistence of the pain following the injection of 3 per cent NaCl suggests that rapid diffusion is of little influence in counteracting the pain-producing quality of the injection.

The variation of limina of K ion in the normal, in the ischemic, and in the worked muscle is not a significant one since the limina were only ob-

tained in 60 mgm. units. It seems probable that the K content of intercellular fluid in muscle is in equilibrium with that in blood serum, which for man averages about 20 mgm. per cent. The muscle as a whole, however, is commonly reported to contain about 400 to 450 mgm. per cent. The relative amount of intercullar fluid is considered to be identical with the chloride space at about 20 per cent (1). Fenn (2) reported that in rats exercised to fatigue the muscles lose 31 mgm. per cent of potassium per 100 grams of muscle. However, these were circulated muscles. If the non-circulated muscle fibers lose 30 mgm. of K per 100 grams of muscle, and if the intercellular fluid is 20 per cent of the muscle, the resulting concentration in the fluid might reach 30 mgm. per 20 grams fluid or 150 mgm. per cent. This rough calculation comes close to the average limen obtained in the present experiments—about 200 mgm. per cent. But when one considers the probability of the potassium ion as factor P, an objection arises in the constancy of the limen which apparently is not reduced by working the ischemic arm.

The lactate ion or its concomitant, the hydrogen ion, has long been assumed to be factor P, though there has been no direct evidence. The elimination of pain within a few seconds by returning blood supply makes it hard to accept the lactate ion as factor P, since lactate diffuses slowly and probably cannot be rapidly oxidized. It is not surprising then that a relatively strong lactate solution does not elicit pain. The maximal lactate ion concentration in fatigued muscle is variously quoted as from 250 to 500 mgm. per cent. No greater concentration would be likely in the intercellular space. The most concentrated solution here tried was 1800 mgm. per cent; at pH 7.0 it was not painful. Nor did it prove effective at pH 4.8, in the few trials made. In previous experiments the pH of the intercellular fluid of this muscle has never been found below pH 6.5 after painful ischemic work (7b). Hence it seems also improbable that the combination of H^+ and lactate ion concentrations can be factor P.

The most likely source for the ammonium ion in muscle working ischemically is the deamination of adenylic acid. The concentration of adenosine triphosphate in mammalian muscle is given as 0.25 per cent. If this entire quantity were deaminated the resulting concentration of ammonium ion would not exceed 10 mgm. per cent.

Several mechanisms suggest themselves which might enable one of these ions to be factor P. If there were a concentration of the released ion around certain nerve endings; if the nerve endings conveying the modality were intracellular rather than extracellular; if the nerve endings were shielded so that the solutions injected intramuscularly were much diluted before reaching them—any of these possibilities might rationalize these limina with the possibility that one of these ions may be factor P. None of these seems probable.

Finally, it may be that the modality evoked by injection is not the same as that produced by ischemic work. Thirty-three per cent of the subjects called the sensations qualitatively different. However, I have experienced pain produced each way many times and I am convinced that only one modality is thus elicited. Sir Thomas Lewis (6) and his more carefully selected subjects were likewise firmly convinced that but one modality exists whether evoked by injection or by ischemic work.

The list of the products of contraction is by no means exhausted with these three ions. Acetyl choline should be tested further. Adenylic acid, inosinic acid, and histamine are likely possibilities as yet untried.

These results show that solutions for intramuscular therapy must contain less than 200 mgm. per cent K ion, less than 200 mgm. per cent NH_4 ion if they are to be painless.

The author wishes to express his gratitude to the medical students of St. Louis University for their generosity in volunteering as subjects for these experiments.

SUMMARY

Since the ischemic pain modality can be evoked by injection of solutions intramuscularly it was thought possible to seek the muscle catabolite which causes the pain in ischemic work by determining the liminal concentration of ions administered intramuscularly. Theoretically the culpable ion should show a limen within the range of physiological production and the limen should be reduced in the ischemic muscle worked to a degree incapable of producing pain.

Accordingly limina were determined in the normal muscle and the ischemic worked but not painful muscle for the K, NH_4 and sarcolactate ions. The latter 2 ions were found to have limina far above the known physiologic maxima. None of the three limina were reduced by ischemic work. It is concluded that if the culpable factor acts on nerve fibers in the intercellular space it is improbable that the sarcolactate ion, the NH_4 ion or the K ion is the pain factor.

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DEPRESSION OF EXPERIMENTAL POLYCYTHEMIAS BY CHOLINE HYDROCHLORIDE OR LIVER ADMINISTRATION

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Received for publication June 30, 1939

In 1938 the author (1) showed that the feeding of raw hog or beef liver caused a prompt reduction in the erythrocyte numbers of dogs which had been made polycythemic by regular daily treadmill running, or by the feeding of cobaltous chloride. It was also found that the injection of one liver extract (Valentine's parenteral) was effective in depressing hematopoiesis, but that the feeding of another extract (Valentine's oral) or of ventriculin was ineffective in this respect.

Recently, Jacobs (2) has reported that liver extract (Lilly, for oral use) contains at least one per cent of choline.

This report led us to the present investigation which deals particularly with the effect of orally administered choline hydrochloride upon experimental polycythemia in dogs, some of the results of which have been published earlier in a preliminary paper (3).

PROCEDURE. Seven dogs were used in these studies. After stabilization on a constant adequate diet of purina dog chow, these dogs were trained to lie quietly while blood samples were withdrawn from the saphenous vein. The normal erythrocyte numbers, hemoglobin percentages (Sahli), total leukocyte counts, and reticulocyte percentages by the method of Wakerlin (4) were determined over periods of 2 to 6 weeks. The values for these blood components were then followed at frequent intervals throughout the experiments.

Polycythemia was produced in 4 dogs by placing them for 6 hours daily in a low pressure chamber[1] in which the environmental air pressure was reduced to about 430 mm. of mercury by a motor driven vacuum pump. It was found that 2 to 3 weeks of this program produced chronic increases of 20 to 30 per cent in the *basal* or *resting* erythrocyte numbers.

In a second series of 3 dogs, polycythemia was produced by the daily oral administration of 2 mgm. of cobalt per kilogram of body weight in

[1] The author's gratitude is expressed here to Dr. A. J. Carlson of the University of Chicago, for the loan of the pressure chamber and vacuum pump used in this work.

the form of a solution of cobaltous chloride. This procedure caused a definite increase in the *basal* erythrocyte numbers, hemoglobin, and reticulocyte percentages—changes which we have previously shown (5, 1) to occur usually in about 2 weeks. Cobalt polycythemia was first demonstrated by Waltner (6) in rats.

After inducing experimental polycythemia by these two methods, we gave the dogs 8 mgm. of choline hydrochloride (Eastman) per kilogram of body weight per day, in 1 per cent solution by stomach tube, for periods of 6 to 8 days; and simultaneously continued the hematopoietic-stimulating routine. Choline hydrochloride was also given to four normal, untreated,

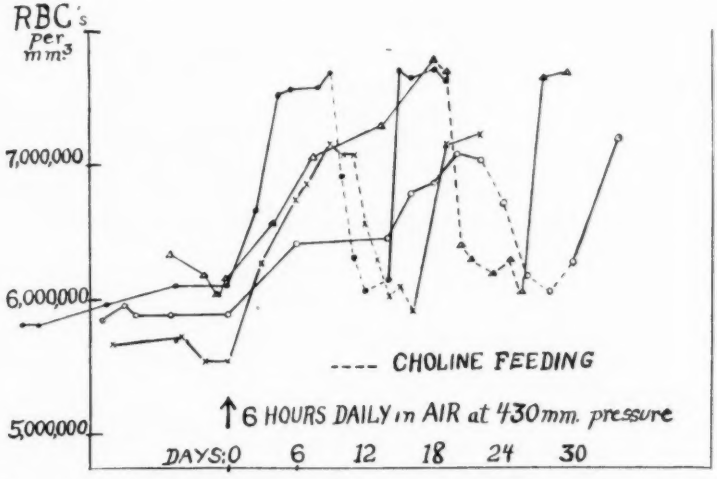

Fig. 1. The effect of choline hydrochloride upon the red blood cell counts of four dogs which were made polycythemic by daily exposure to reduced atmospheric pressure.

dogs for a similar duration of time, to see whether the normal red cell count would be affected thereby.

In the liver feeding experiments, 75 grams per dog were given daily as in our previous work (1), and the liver was raw unless otherwise stated.

Results. Figure 1 shows the changes in red cell counts of 4 dogs in which erythropoiesis was stimulated by exposure of the dogs for 6 hours daily to an environmental air pressure of 430 mm. The erythrocyte numbers were increased by 19 to 30 per cent within 8 to 18 days by this procedure. When choline hydrochloride was administered daily, the red cell counts dropped promptly (after 2 or 3 doses) approximately to their normal values and remained depressed, in spite of daily exposure to low

air pressure, for the entire duration of choline feeding. Upon discontinuation of choline, polycythemic values were again obtained. The percentage

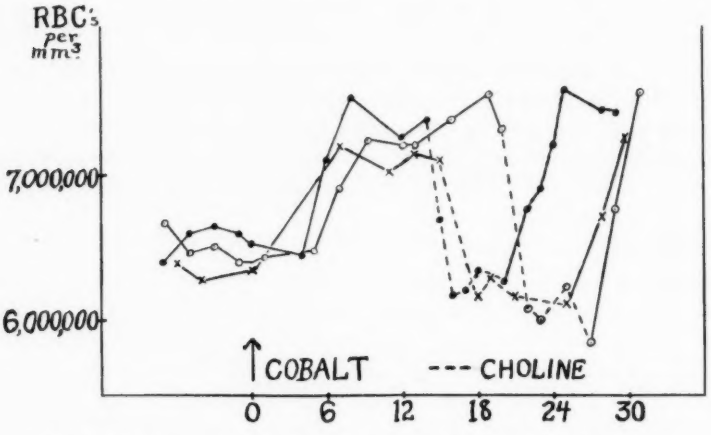

Fig. 2. The depression of cobalt-induced polycythemia in three dogs

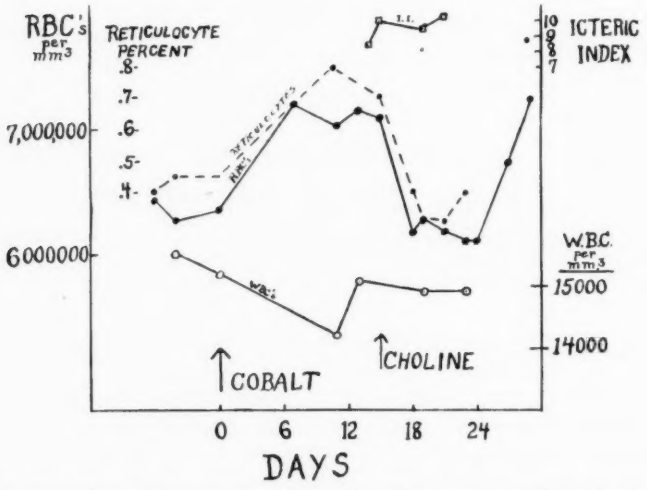

Fig. 3. The effect of choline upon various blood components of one dog in which polycythemia was induced by cobalt.

of hemoglobin in the circulating blood (not shown) varied proportionally with the erythrocyte numbers. Reticulocyte percentages were at least doubled by the low pressure regime, but were depressed to or below normal

during the periods of choline feeding. Total leukocyte numbers remained fairly constant throughout the entire series of experiments, rarely varying by more than 10 per cent.

The production of polycythemia by cobalt feeding and its subsequent reduction by choline administration in 3 dogs is shown in figure 2. Here, again, reticulocytosis occurred with the polycythemia, but was depressed

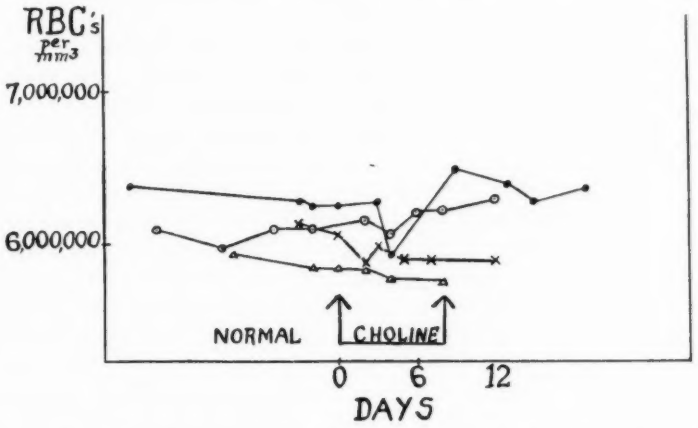

Fig. 4. The effect of choline hydrochloride upon the normal erythrocyte numbers of four normal, untreated dogs.

TABLE 1

Effect of raw and boiled liver upon polycythemia

Erythrocyte numbers are given in millions per cubic millimeter

DOG NUMBER	LIVER RAW OR BOILED	POLYCYTHEMIC VALUE	AFTER 1 DAILY DOSE	AFTER 2 DAILY DOSES	AFTER 3 DAILY DOSES	AFTER 4 DAILY DOSES
1	Raw	7.73	7.18	6.51	6.23	6.35
4	Raw	7.19		6.20	6.04	6.18
6	Raw	7.61	7.54	7.01	6.66	6.17
5	Boiled	7.23	6.93		7.05	6.96
6	Boiled	7.68		7.45	7.61	7.54

with the erythrocyte numbers when choline was fed, while the total leukocyte counts did not change significantly. Although the leukocyte counts and reticulocyte percentages are not shown in figure 2, the values for these components in one cobalt-fed dog are shown in figure 3. Several determinations of the icterus index were made on 2 dogs just before, and early in, the period of choline feeding, and no significant changes were observed.

The feeding of choline hydrochloride to 4 *normal*, untreated dogs (fig. 4) did *not* cause any significant change in their normal erythrocyte numbers.

Seventy-five grams of raw beef and hog liver were fed daily for 4 days to 3 of the polycythemic dogs which were exposed to low atmospheric pressure. This caused a prompt reduction of their erythrocyte numbers to or below normal. The results (see table 1) show that raw liver reduces this type of polycythemia, confirming our previously published results (1) on cobalt and exercise polycythemias.

The same quantity of liver, boiled in water for 30 minutes, was fed together with the added water, to 2 polycythemic dogs, and this treatment *failed* to reduce the erythrocyte numbers significantly (see table 1).

Discussion. The results of these experiments indicate that the oral administration of 75 mgm. of choline hydrochloride is approximately as effective as the feeding of 75 grams of *raw* liver (see previous paper (1)) in depressing experimental polycythemia in dogs. Both liver and choline appear to act by depressing hematopoiesis when the red blood cell count is unusually high, as judged chiefly by the depression of reticulocytosis. It is not within the domain of this discussion to speculate as to whether choline acts directly upon the hematopoietic tissue, or indirectly through the liver, or conceivably through some rôle in fat metabolism.

The active liver constituent which depresses polycythemia is probably choline. At least it is heat labile, and probably not identical with any anti-anemic principles, as shown by our previous finding (1) that ventriculin and anti-anemic liver extract (heated in preparation) were ineffective in polycythemia.

The report of Major (7) that liver extract is without effect on the red blood cell count of patients suffering from polycythemia vera, is not surprising, in view of the various methods by which liver extracts are prepared, and the ease with which the important principle or principles might be lost.

Since choline is present in liver, and since we have shown that choline depresses polycythemia in dogs, it seems possible that choline, in moderate doses, might be of value in the treatment of polycythemia vera.

CONCLUSIONS

The oral administration of 8 mgm. of choline hydrochloride per kilogram of body weight per day to polycythemic dogs *depresses* hematopoiesis and tends to return the erythrocyte number to normal.

The feeding of *raw* hog or beef liver in doses of 75 grams per day to dogs *reduces* the polycythemia caused by daily exposure to low atmospheric pressure under the conditions of these experiments, as well as that caused by exercise or cobalt.

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SEASONAL VARIATION IN THE RESPONSE OF FROG BODY WATER TO PITUITRIN[1]

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Received for publication June 9, 1939

During the past three years, experiments have been performed in this laboratory on the action of pituitrin upon the body water of frogs. This work has been done chiefly during the early spring to early autumn season of the year. In the session 1938–1939, the experiments were continued for the first time throughout an entire year. In late October and November of 1938 the response of stored frogs to pituitrin began to decline. Whereas in the summer, frogs immersed in water had taken up, on the average, about 20 per cent of their weight of water in 3 to 5 hours after injecting sufficient pituitrin, in the late autumn the uptake began to decrease. The present report is concerned with a record of the reaction as obtained at different seasons of the entire year together with experiments designed to explain certain features of the seasonal variation.

Leopard frogs (Rana pipiens) were used and stored in tanks with running water. The general technique employed was similar to that reported by Boyd and Brown (1). The dose of pituitrin used for a seasonal comparison was, except as otherwise noted, 0.2 international unit of Pituitrin Surgical per 10 grams body weight of frog.

To demonstrate the effect of season, the maximal uptake of water was selected as an index of the reaction to pituitrin. Representative experiments with 10 to 20 frogs each were then selected from our records at different months of the year and the mean, maximal uptake of water calculated as a percentage of the initial weight before injecting pituitrin. Any changes in the controls not receiving pituitrin were then subtracted from this figure and a number of the resulting values have been given in table 1. It is obvious from these results that pituitrin caused the greatest uptake of water in the seasons between late spring and early autumn. The response then began to decline, reached its lowest values in mid-winter and then rose again to the summer peak in the spring. The remaining

[1] Pituitrin Surgical was generously supplied by Dr. E. A. Sharp of Parke, Davis and Company, which Company also kindly provided a grant which defrayed part of the expenses of this work.

experiments were concerned with factors which might have affected this seasonal variation.

Storage. Storage was not the factor responsible for the seasonal change because the recovery in the late spring was obtained with frogs which had been stored all winter as well as in fresh frogs captured in their natural habitat.

Control frogs. The results shown in table 1 represent mean changes in controls not receiving pituitrin subtracted from mean changes in pituitrin-injected frogs. During the summer, variation in the water content of control frogs used during the experiment were found insignificant, a finding also recorded by Boyd and Brown (1). In the cold seasons of the year, however, frogs brought up from the storage tanks in the basement consistently lost weight during the experiment and this decrease in weight averaged 2 to 3 per cent per hour in mid-winter.

TABLE 1

The mean, maximal percentage uptake of water by frogs immersed in water and injected with pituitrin at succeeding seasons of the year

DATE	MAXIMAL PERCENTAGE UPTAKE	DATE	MAXIMAL PERCENTAGE UPTAKE
June 20, 1938	19.0	January 28, 1939	10.5
July 15, 1938	21.3	February 16, 1939	11.7
August 2, 1938	19.3	March 2, 1939	13.2
August 31, 1938	19.2	April 24, 1939	13.5
September 16, 1938	20.4	May 6, 1939	16.2
October 15, 1938	15.0	May 13, 1939	23.0
November 5, 1938	12.1	May 19, 1939	20.8
December 2, 1938	10.5	May 27, 1939	25.2

Investigating the reason for this loss of weight in controls, it was noted first that the temperature of the running water in the storage tanks was about 6°C. in mid-winter. The running water came from Lake Ontario which in this area is covered with ice in mid-winter. It seemed possible that the change in temperature from 6°C. to room temperature of 22 to 23°C. might have been a factor. Hence a group of frogs was stored in aquaria at room temperature. This resulted in a decreased loss in weight of controls but even after several weeks of storage at room temperature the controls still lost weight as handled during the course of an experiment. Part, but not all, of the loss in weight of controls has thus been accounted for. Jones and Steggerda (2) reported a loss of water in control frogs during experiments performed in February and March at Urbana, Illinois.

It so happened that one of us was interested in determining the reaction of frogs to pituitrin at 4°C. (the temperature of the refrigerator room) during the same seasons as those in which the above experiments were

being done. In this latter work, the entire reaction to pituitrin was determined in the refrigerator room. The maximum response at 4°C. was the same as that at room temperature but the entire reaction was slowed so that more frogs could be simultaneously used for assay purposes which was the reason for this study. It had been found in the summer months that when frogs were changed from storage at room temperature to storage at 4°C. they took up 10 to 15 per cent of their weight of water. Conversely, when removed from storage at 4°C. to room temperature, they lost this extra water. This change in normal body water explained further a part of the loss in water of controls brought to room temperature from the storage tanks in mid-winter, as described in the previous section.

There remained to explain the further loss of water of controls acclimatized to room temperature in mid-winter. Evidently this was due to handling, but why handling should cause frogs to lose water in the winter and not in the summer was not found. It was noted, however, that when the entire experiment was performed at 4°C., the controls did not lose water during an experiment at any season of the year. Apparently handling frogs in the winter months results in a loss of water at room temperature but at no season results in a loss of water at 4°C. This may be related to some neuro-endocrine reaction, insignificant at 4°C. but effective at room temperature, operative only in the winter seasons and perhaps associated with ova production in the female and some corresponding change in the male, as suggested below.

Temperature. The decreased response to pituitrin in the hibernating seasons for frogs might have been due to prolonged storage at low temperatures. This did not appear to be the responsible factor because frogs stored in the refrigerator at 4°C. all summer gave the same response to pituitrin as frogs stored at room temperature. In fact the absolute response of frogs stored in the refrigerator room was the same the year round as that of frogs stored in the basement tanks with running water. Further, frogs stored in the refrigerator room and then acclimatized to room temperature for a few days gave the same response to pituitrin as frogs stored in the basement tanks and similarly acclimatized. These results indicate that the continuous low temperature of storage during the hibernating seasons could not have been the factor responsible for the diminished response to pituitrin in the winter.

Light. The daily quota of sunlight is longer in summer than in winter but this did not appear to have been a factor in causing the seasonal changes noted because the frogs were stored in a dark, windowless (apart from a small ventilating aperture) basement and exposed to light for 6 to 8 hours each day an experiment was run, summer and winter.

Dosage of pituitrin. It was possible that during the winter season frogs required more pituitrin in order to yield the maximal response. When

this was investigated, it was found that the *relative* response of frogs to doses of pituitrin varying between 0.01 and 1.0 international unit per 10 grams body weight was the same in winter as in summer. That is to say, the maximal response to pituitrin was obtained with 0.1 unit in the summer and this same dose gave the maximal response in the winter. In no instance did a dose greater than 0.2 unit produce a response greater than that shown in table 1 at any season of the year. Apparently, then, in the winter frogs are incapable of responding to the same extent as in the summer.

Fatigue. Early in this work it was found that frogs injected each successive day (except Sunday) responded normally for a period of 6 to 8 weeks. Then the response became weak and erratic. If given a rest period of 2 weeks, they again responded normally. These facts were taken into consideration in subsequent work and frogs were not used more often than 1 to 3 times a week, usually one group of frogs was used only once a week. Further, these same frogs gave an increased response in the spring, after low responses all winter, so that fatigue could not have been the factor responsible for the seasonal changes.

Intrinsic factors. The above experiments had eliminated all of the obvious and apparent extrinsic factors which might have been responsible for seasonal variation. Since no extrinsic factor was found responsible, it appeared probable that the seasonal variation was due to some intrinsic factor. The most obvious intrinsic factor suggesting itself is the sexual cycle. Marshall (3) states that oviposition in the frog occurs in March and April and the diminished response at and before this time may have been associated with ova production in the female and perhaps a corresponding change in the male preparing it for the sexual act. Presumably Marshall's date of March–April is for England; the season of oviposition in this district is, so far as the authors could ascertain, unknown. Unfortunately, this idea did not occur to us during the year in which the above data were obtained and so exact information was not tabulated on the sex of the frog, the percentage weight of ova contained in the females, the number of males found in the sexual embrace, the quantity of eggs floating on the water, etc. However, it was recalled that more males were seen in the sexual embrace in the winter than in the summer and that frog spawn appeared on the surface of water in a tank in which frogs were stored with very little running water in April. For the time being and until more accurate data have been collected, this would suggest that the decreased response to pituitrin coincided with the season of greatest sexual activity as evidenced by sexual embraces and ova production and oviposition. It is perhaps further significant that following the period of oviposition in April, the response to pituitrin rapidly increased to the summer maximum.

SUMMARY

A seasonal study of leopard frogs in a water bath revealed that when pituitrin was injected they took up a mean of about 20 per cent of their weight of water in the non-hibernating and of about 10 per cent in the hibernating seasons of the year.

No extrinsic factor studied—storage, light, temperature, fatigue, dose of pituitrin or changes in controls—was found causative of the seasonal change.

It was suggested that the diminished response to pituitrin in the winter may be associated with a sexual cycle.

Another evidence of a seasonal change was that normal frogs lose water when handled in the winter at room temperature but not in the summer.

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THE INCREASED ACCOMMODATION TO ELECTRIC CURRENTS PRODUCED BY VAGAL INHIBITION OF THE TURTLE ATRIUM

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Received for publication June 12, 1939

The changes in excitability of cardiac tissue during a period of vagal inhibition have been interpreted in a variety of ways. Previous experiments have indicated that vagal inhibition results in a shortening of chronaxie (Fredericq, 1924; Lapicque and Veil, 1924; Field and Brücke, 1926). Garrey and Ashman (1931) and Ashman and Garrey (1931), using constant current stimuli, attempted a critical study of the excitability changes reported for the turtle heart during inhibition. For the excitability of the turtle atrium as determined by constant currents, they found in general that vagal inhibition resulted in a rise of the observed rheobase, a shortening of the chronaxie, and a lengthening of the least time required for stimulation by a current of greater than rheobasic strength. They concluded (p. 119) that "If excitability be defined in terms of the quantity of energy required for excitation, then vagus stimulation decreases excitability."

In the course of experiments dealing with vagal action on the turtle atrium, we have repeatedly tested excitability of the atria by means of induction shocks or condenser discharges of brief duration and found that *by this method of measurement* there was no decrease in excitability during periods of inhibition. If changes in threshold have been observed, they have usually been slight and they have shown no consistent directional change. The present investigation was undertaken to determine the relationship existing between the form of the stimulating current used for determining the cardiac excitability and the apparent changes in excitability measured.

METHOD. The atria of turtles, usually P. elegans, were used. Dissections were made to obtain free exposure of the atria, the ventricle usually being cut away. The vagi were freely exposed and cut high in the neck region. Completely excised vagus-heart preparations perfused with oxygenated, bicarbonate buffered saline mixtures were employed in some cases. In most experiments we have used *in situ*, unperfused preparations, in some cases with normal sino-atrial conduction, in other

cases with sino-atrial compression to maintain the atria in standstill. Stimulating electrodes were of the calomel type, the cathodal electrode being connected to the atrium by a saline-soaked yarn which was either tied to or merely placed in contact with the moist surface of the atrium. The anodal electrode was placed in contact with some remote part of the body. Each of the variations in procedure introduced its own advantages and disadvantages. Without discussing each in detail, it may be said that in interpreting the experimental results the attempt has been made to use proper controls against possible complications introduced by the procedures followed.

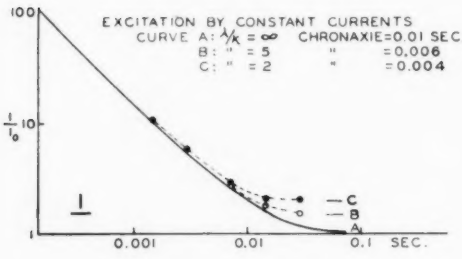

Fig. 1

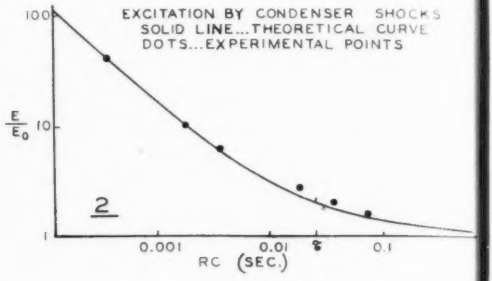

Fig. 2

Fig. 1. Excitation curves according to Hill's treatment indicating the forms of the I-t curves and values of chronaxie and measured rheobase for a hypothetical preparation showing, in A, no accommodation, and in B and C rapid accommodation to applied currents. In B and C, the rheobase is elevated but the threshold to shocks of very brief duration remains unchanged.

Fig. 2. "Normal excitability." Data from experiment on turtle atrium (dots) plotted in relation to the theoretical curve (solid line). Vagus inhibition causes a change corresponding to the change from curve A to curve B of figure 1.

As a source of stimulating current, a device was arranged to give, through a single pair of stimulating electrodes, either constant currents, series-condenser discharges of the usual form, or currents rising exponentially to a level plateau. For the last type, exponentially rising currents, a parallel-condenser arrangement was used as a control in a few experiments. In most experiments this was replaced by a more convenient arrangement using as a source of stimulating current the plate current of a triode tube (type 89) while a negatively charged grid condenser was discharged through a known resistance. The current so produced yielded experimental data practically identical with those obtained with the simple parallel condenser circuit. We shall therefore refer to both these stimulus types as the "exponentially rising currents."

RESULTS AND DISCUSSION. During vagal stimulation, the atrium showed

an elevation of rheobase and of threshold to the "exponentially rising currents." There was no significant change of atrial threshold as measured by short condenser shocks. Irregularities of the sort noted by Ashman and Garrey were seen. In general it appeared that the better the condition of the preparation, the less was the tendency for such irregularities to appear.

The theoretical treatment of excitation and accommodation used by Hill (1936) has been found convenient in the description of the changes in cardiac excitability which have been observed with the various shock forms employed. The effect on excitability produced by vagal inhibition can be interpreted as due to a change in the amount of accommodation which may be developed during the passage of a stimulating current. Such an interpretation is in harmony with the facts of most of the previously published material, especially that of Ashman and Garrey. Assuming, for the moment, complete agreement of the Hill treatment with the facts of cardiac excitability, we have plotted, as figure 1, the theoretical current-time curves for a preparation showing (A) no accommodation and (B and C) rapid accommodation. Specific time values have been used for plotting and were assumed from the data of the experiment yielding the points of figure 2. It will be seen by comparison of the curves of figure 1 that for the current-time values reported by Ashman and Garrey, a preparation which developed a significant increase of accommodation to a stimulating current applied during vagal inhibition would demand a greater least time for a given current strength, a finding which they emphasize. If accommodation were the only aspect of the excitability complex to be changed during the period of inhibition, the atrial threshold to very short shocks would be unchanged. Our failure to observe significant change of atrial threshold when using very short duration shocks indicates that this is actually what occurs.

Excerpts from a single experiment are given as an example of one type of procedure which has been followed. Using a constant current for rheobase determinations, measuring both current and voltage, and using condenser stimulation for other determinations of threshold, the data plotted as figure 2 were obtained. A value for κ (Hill) was determined by fitting these points to his theoretical curve which is drawn as the solid line of figure 2. For the sake of consistency, chronaxie was calculated using the value 0.347 RC rather than the value of 0.37 RC. Since the plotted experimental points all indicate responses, the theoretical curve is placed to fall below all experimental points rather than through their mean distribution.

Using "exponentially rising currents," a normal value for $\frac{\lambda}{\kappa}$ was obtained. The normal values thus found were: $-\kappa = 12.8$ msec., chronaxie =

8.87 msec., $\lambda = 455.$ msec., $\frac{\lambda}{\kappa} = 36.$ The last value is less than that usually observed in a perfused, oxygenated preparation but is fairly typical of the unperfused preparation. A condenser shock having an RC product of 0.26 msec. required 84 volts for response. Stimulation of the left vagus (2 volts, 60 cycle) with no rate change in the heart resulted in an increase of the threshold condenser shock to 85.5 volts, an increase of about 2 per cent. The rheobase was, however, increased by 45 per cent. The succeeding period of right vagal stimulation caused standstill of the heart, a decrease of the condenser shock threshold of 4 per cent and an increase of rheobase by 35 per cent. During one period of inhibition, use of "exponentially rising currents" yielded data for determining $\frac{\lambda}{\kappa}$ (Solandt, 1936) as approximately 12, indicating a much more rapid accommodation to a stimulating current than the normal. The extent of the vagal inhibition chosen in this case was such that the contraction of the atrium during the period of inhibition was slight and was definitely visible. During the periods of vagal stimulation, paired excitation determinations such as those presented above have been used as it has not been possible to obtain good data for a complete curve such as that used for the "normal" excitability plotted in figure 2.

Whether there is a quantitative relationship between the change in the development of accommodation to the stimulating current and the degree of inotropic inhibition produced in the atrium has not been determined. Neither is it yet possible to state the maximum extent to which the rate of accommodation may be developed in a still responsive atrium. Two experiments on the right atrium with right vagus stimulation showed apparent $\frac{\lambda}{\kappa}$ values as low as 5, which indicates great accommodation of the atrium to applied currents. In both these cases, however, the right atrial movement was so slight as to make possible the interpretation that there was remote stimulation of the less inhibited left atrium with no proper response of the right atrium. The $\frac{\lambda}{\kappa}$ ratio of 2 used in plotted figure 1C has never been observed in our experiments. It is demanded under the Hill treatment for a preparation showing a doubling of the rheobase or a reduction of the chronaxie to 0.42 times that for the non-accommodating preparation.

In conclusion, it may be said that we believe our findings confirm but extend the experimental findings of Ashman and Garrey. It is only by using the shorter time values and correspondingly higher stimulus strengths that the thresholds of the normal and vagal inhibited preparations show the same responsiveness to stimulation.

The changes in the course of recovery following a beat of the atrium have been considered previously (Gilson, 1935) in papers which have pointed out the importance of taking into account the form of the shock used for measuring thresholds. The present paper indicates that it is similarly important to consider the shock form in determinations of resting or late diastolic excitabilities. Since the time functions of the propagated action potential of the heart do not fall into the class of the "very short shock," the elevation of rheobase and the shortening of chronaxie observed during a period of vagal inhibition may well be regarded as indicative of a general decrease in the functional excitability of the preparation.

SUMMARY

1. The previously reported increase of rheobase and shortening of chronaxie during vagal inhibition of the turtle atrium is confirmed.

2. The use of constant currents and of currents rising slowly to a constant level indicate that vagal inhibition may produce changes in the atrium such that it shows a great increase in its accommodation to applied stimulating currents.

3. Measurements of excitability using induction shocks or condenser discharges of brief duration do not show a consistent elevation of threshold during vagal inhibition.

4. The experimental findings are compatible with the interpretation that the observed changes in excitability of the vagus inhibited turtle atrium result from an increased accommodation to the stimulating currents.

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DETERMINATION OF THE TOTAL CHLORIDE CONTENT OF ANIMALS FOLLOWING ADMINISTRATION OF SODIUM BROMIDE

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Received for publication June 19, 1939

Previous work has shown that following the administration of sodium bromide either by mouth or intravenously the replacement of chloride by bromide expressed as the ratio $\frac{(Br)}{(Cl+Br)}$ is a constant for blood and all other tissues examined (muscle, skin, bone, viscera) nervous tissue and spinal fluid excepted (1, 2).

Provided therefore that a known amount of bromide is injected into an animal, that time is allowed for the bromide to equilibrate itself between blood and tissues and that precautions are taken to prevent loss of bromide from the body during the period of equilibration, one should be able to accurately estimate the total chloride content of the animal from a blood analysis of chloride and bromide content. The following report is based upon this premise.

METHODS. Experiments were performed on dogs, cats and rabbits. Following anesthetization with dial-urethane the kidneys were exposed and tied off to prevent loss of chloride or bromide in the urine. Subsequent leakage of fluid from the peritoneal cavity was carefully guarded against. The injections of sodium bromide were made into the exposed femoral vein through a hypodermic needle-burette system.

Cats and rabbits received 15 to 20 cc. of a solution containing 12 to 15 grams NaBr/100 cc. H_2O. The volumes of the solutions injected and the bromide content of the solutions were accurately measured.

Into the dogs was injected an accurately weighed quantity of NaBr (10–15 grams) dissolved in a minimum amount of water. The flask in which the NaBr was dissolved as well as the burette through which the injections were made was then washed two or three times with small amounts of distilled water. These washings were also introduced into the animal through the burette.

Following the equilibration period, a sample of blood was drawn under

oil from either the heart (cats and rabbits) or the femoral artery (dogs) and centrifuged. The serum obtained was used for analysis.

Chemical studies involved the determination of bromide and total halide.

Bromide. The potentiometric method of Hastings and Van Dyke (3) was employed in the determination of bromide. In preparing the serum for the titration, one cubic centimeter samples were pipetted into porcelain crucibles containing 0.5 gram of a 2:1 mixture of $Ba(NO_3)_2$ and Na_2CO_3. The serum salt mixtures were evaporated to dryness in an oven and then placed overnight in a muffle furnace set for 420°C. Preliminary studies showed that no loss of bromide occurred with this ashing mixture at the temperature employed. In our hands, the mixture proved far superior to the use of concentrated KOH which has been recommended for this purpose.

When ashing was complete the contents of the crucible were washed quantitatively into 100 cc. volumetric flasks, neutralized with HNO_3 using methyl orange as indicator and diluted to the mark with distilled water. Fifty cubic centimeters of the solution representing 0.5 cc. of serum were used for a titration. All determinations were run in duplicate.

Total halide (and chloride). Total halides were determined by the wet ash method of Van Slyke and Sendroy (4). Chlorides were determined as the difference

(total halide) − (bromide) = (chloride)

Results. In tables 1, 2 and 3 are presented the results obtained upon a series of dogs, cats, and rabbits respectively. Although the average values obtained for the chloride content of these animals expressed as grams chloride per kilogram of body weight are of the same order of magnitude (dogs = 1.18; cats = 1.22; rabbits = 1.07), it appears that the chloride content of rabbits is, on the average, definitely lower than that of dogs and cats.

The question whether the method described for determining the total chloride content of animal is valid lends itself to experimental test. Following the bromide equilibration period and withdrawal of the sample of blood for analysis, five grams of chloride (as sodium chloride) were injected into a dog (see dog 5 a, b; table 1). One hour later a second sample of blood was withdrawn for analysis. The results of analysis of the second sample showed that the chloride content of the animal had increased from 16.64 grams to 21.42 grams or by 4.78 grams. In other words 95.6 per cent of the added chloride was detectable by the method used.

In dog 6 (a, b, c, d) the effect of the time allowed for equilibration is shown. Equilibrium is attained within 50 minutes. In all experiments reported the time allowed for equilibrium has been from 60 to 90 minutes.

Discussion. Estimations of the total chloride content of animals have been made in the past. The technique used has been either to extract the

TABLE 1

Summary of results obtained on a series of dogs

DOG NUMBER	[Cl⁻ + Br⁻]	[Br⁻]	[Cl⁻]	[Cl⁻]/[Br⁻]	MOLS Br⁻ GIVEN	MOLS Cl⁻ (CALC.)	Cl⁻	WEIGHT	Cl⁻ PER KGM.	REMARKS
	Millimols per liter						*grams*	*kgm.*	*grams*	
1	129.5	62.2	67.3	1.08	0.286	0.333	11.7	10.95	1.00*	
2	138.0	52.6	87.4	1.66	0.272	0.504	17.86	12.55	1.42*	
3	140.5	41.6	98.9	2.38	0.194	0.462	16.38	11.8	1.39	
4	145.0	54.0	91.0	1.685	0.194	0.327	11.64	9.7	1.20	
8	130.8	30.4	100.4	3.30	0.146	0.482	17.09	13.85	1.23	
9	137.6	44.0	93.6	2.13	0.146	0.312	11.06	10.27	1.07	
10	127.8	27.0	100.8	3.735	0.147	0.551	19.55	18.37	1.06	
5a	133.2	39.0	94.2	2.415	0.194	0.469	16.64	13.75	1.21	Then 5.00 grams Cl⁻ intravenously
5b	147.9	36.0	111.9	3.11	†	0.604	21.42			4.78 grams Cl recovered
6a	133.8	42.2	91.4	2.165	0.194	0.421	14.95	14.27	1.05	Time, 50 minutes
6b	132.5	42.8	89.7	2.10	†	0.408	14.45	14.27	1.01	Time, 80 minutes
6c	135.3	41.6	93.7	2.25	†	0.438	15.52	14.27	1.09	Time, 112 minutes
6d	141.9	43.2	98.7	2.28	†	0.445	15.77	14.27	1.10	Time, 145 minutes
Average									1.18	

* Data of dogs 1 and 2 corrected for urinary excretion.

† No additional.

TABLE 2

Summary of results obtained on a series of cats

CAT NUMBER	[Cl⁻ + Br⁻]	[Br⁻]	[Cl⁻]	[Cl⁻]/[Br⁻]	MOLS Br⁻ GIVEN	MOLS Cl⁻ (CALC.)	Cl⁻	WEIGHT	Cl⁻ PER KGM.
	Millimols per liter						*grams*	*kgm.*	*grams*
1	132.7	36.2	96.5	2.67	0.0277	0.0737	2.61	2.15	1.22
2	141.9	32.6	109.3	3.35	0.0251	0.0843	2.99	2.15	1.39
3	133.3	28.0	105.3	3.76	0.0234	0.0882	3.15	2.86	1.09
4	152.8	46.4	106.4	2.29	0.0228	0.0522	1.85	1.82	1.02
5	160.3	33.2	127.1	3.82	0.0232	0.0889	3.15	2.17	1.45
6	131.4	26.4	107.7	4.08	0.0137	0.0558	1.98	1.62	1.22
7	135.5	24.8	110.7	4.47	0.0137	0.0611	2.17	1.50	1.44
8	138.0	35.4	92.6	2.61	0.0191	0.0499	1.77	1.82	.97
9	130.4	30.4	100.0	3.29	0.0264	0.0868	3.08	2.90	1.06
10	133.8	27.6	106.2	3.85	0.0264	0.102	3.61	2.60	1.39
Average									1.22

entire animal with alkali and analyze the extract for chloride or to grind the entire animal and analyze a portion of the homogenized material for chloride.

Using the former method Rosemann (5) reported in a series of three adult dogs values of 1.05, 1.19, and 1.36 grams of chloride per kilogram body weight.

The technique of analyzing homogenized aliquot portions of animals has recently been employed by Harrison, Darrow and Yannet (6) to study, among other body constituents, the total chloride content of dogs, rabbits and monkeys. Recalculation of their data to express the chloride content in terms of grams of chloride per kilogram body weight, reveals in a series of two dogs values of 1.13 and 1.27. Similar recalculations upon a series of three rabbits reveals values of 1.14, 1.10 and 1.06 grams chloride per kilogram body weight.

TABLE 3

Summary of results obtained on a series of rabbits

RABBIT NUMBER	$[Cl^- + Br^-]$	$[Br^-]$	$[Cl^-]$	$\frac{[Cl^-]}{[Br^-]}$	MOLS Br^- GIVEN	MOLS Cl^- (CALC.)	Cl^-	WEIGHT	Cl^- PER KGM.
	Millimols per liter						*grams*	*kgm.*	*grams*
1	121.4	30.2	91.2	3.02	0.0171	0.0517	1.83	1.55	1.18
2	122.2	29.2	93.0	3.18	0.0172	0.0548	1.94	1.38	1.40
3	125.2	37.4	87.8	2.35	0.0172	0.0404	1.43	1.40	1.02
4	126.7	30.8	95.9	3.11	0.0137	0.0426	1.51	1.40	1.08
5	127.7	46.0	81.7	1.775	0.0228	0.0405	1.43	1.32	1.08
6	121.5	32.6	88.9	2.73	0.0291	0.0794	2.81	2.97	0.96
7	121.5	39.6	81.9	2.07	0.0286	0.0593	2.10	2.77	0.76
8	128.3	34.0	94.3	2.77	0.0275	0.0762	2.70	2.37	1.14
9	123.0	27.0	96.0	3.56	0.0285	1.013	3.59	3.14	1.14
10	123.1	31.6	91.5	2.895	0.0221	0.0642	2.27	2.40	.95
Average									1.07

The results presented in this report are seen to be in close agreement with those reported using the more direct methods of analysis.

Furthermore, the method employed has, we feel, the following advantages over previous methods.

1. It is applicable to any sized animal from which it is possible to obtain as little as three or four cubic centimeters of serum.

2. It is not time consuming.

3. The animal utilized need not be sacrificed. In the animals presented in this paper (except dogs 1 and 2) the kidneys were tied off and the animals sacrificed at the termination of the experiment. An alternate procedure is to quantitatively collect the urine excreted during the period of equilibration, analyze the urine for total chloride and bromide content and make the appropriate corrections.

Examination of the data presented shows no inconsiderable variation in chloride content even within a given species of animal. The reason for

this variation is not clear. As is well known, the chloride content of skin is high. This fact suggests that the results obtained may have been more consistent if they had been expressed in terms of surface area. It is equally possible however that the variations are due to differences in fat content of the animals studied. This latter possibility gains support from the fact that of the two dogs studied by Harrison et al. the dog whose total fat content was highest contained less chloride per kilogram body weight than the other dog which contained considerably less body fat. Furthermore when one recalculates the chloride content of these dogs on a fat-free basis the chloride content of both animals is practically identical.

CONCLUSIONS

A method is presented for determining the total chloride content of animals through intravenous injection of sodium bromide.

The chloride content of dogs, cats, and rabbits expressed as grams of chloride per kilogram body weight was found to be 1.18, 1.22 and 1.07 respectively.

The author wishes to acknowledge the technical assistance of P. L. Elmore and F. E. Roberts.

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ACETYLCHOLINE-EQUIVALENT CONTENT OF THE UTERUS AND PLACENTA IN RABBITS[1]

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Received for publication May 13, 1939

No acetylcholine has been found in the placenta of the rabbit (Chang and Gaddum, 1933), although this substance is found in relative abundance in human placental tissues (Chang and Gaddum, 1933; Chang and Wong, 1933; Haupstein, 1932; Strack and Loeschke, 1931). The question of the acetylcholine-equivalent content of the placenta of the rabbit, along with similar determinations on the uterus of the gravid and pseudopregnant rabbit, was investigated in the present work because tissues were readily available from animals employed in another series of experiments.

The rabbits used were of mixed stock, bred in our laboratory from single matings after a period of isolation. The rabbits in oestrus were either recently post partum, or were killed within an hour after mating. Tissues were taken on the sixteenth, the twenty-second, the twenty-eigth and the thirty-first days of gestation. While pregnancy in the rabbit usually comes to an end on the thirty-second day, there is a normal range of variation extending from a little over thirty-one to thirty-four days. This period of variation is governed largely by the physical conditions created in each pregnancy as a result of varying degrees of uterine distention (Reynolds and Foster, 1939; Wishart and Hammond, 1933). In addition to determinations made on these days of pregnancy, a few determinations were made on uteri of rabbits in pseudopregnancy (sixth and sixteenth days).

The method of extracting the tissues was essentially that of Chang and Gaddum (1933) with the minor modifications made by one of us, as noted in an earlier paper (Reynolds, 1939a). The only difference between the procedures employed in this work and the earlier study is that the pressure used for evaporation of the extracts in this work was about 0.1 mm. Hg, while in the earlier study it was about 50 mm. Hg. With the lower pressure and a somewhat shorter period for evaporation, it seems that a consistently higher yield of acetylcholine is obtainable.

The method of testing the extracts was limited to use of the rectus

[1] Supported by grants from the Committee for Research in Problems of Sex, National Research Council, and from the Committee on Scientific Research of the American Medical Association.

abdominis muscle of the frog, precisely as prescribed by Chang and Gaddum (1933), since this preparation is sufficiently sensitive both before and after eserinization (to 0.02–0.20 gamma of acetylcholine); the muscle relaxes well, and the confirmatory test of potentiation of the action of acetylcholine by eserinizing the muscle is easily performed. While this confirmatory test (shown in fig. 1) is not wholly specific for acetylcholine when used alone, it is the most conclusive of the several confirmatory tests available and it may be taken as reasonably good evidence that one is dealing with acetylcholine when potentiation is clearly demonstrable (see Chang and Gaddum, 1933). Even so, since only this one test was employed, reference is made in this paper, as in that of Chang and Gaddum, to the acetylcholine-equivalent content of the tissues studied.

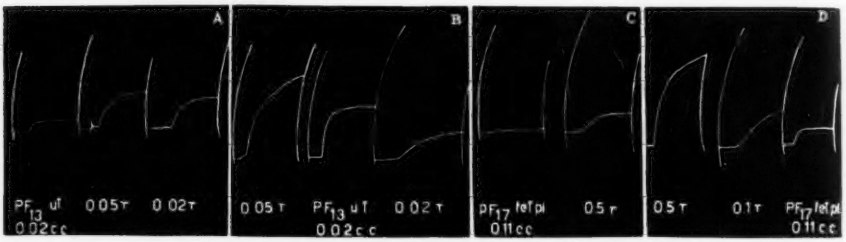

Fig. 1. Records of the action of acetylcholine and of extracts of uterus on the rectus abdominis muscle of the frog; A and C, uneserinized; B and D, after eserinization. A and B, uterine extract prepared on the sixth day of pseudopregnancy; C and D, fetal placenta prepared on the twenty-eighth day of pregnancy. Similar records of eserine potentiation, characteristic of acetylcholine, were obtained with extracts for each day of pregnancy studied, with preparations made from both the fetal placenta and the uterus.

Results. The results of this study are summarized in figure 2. The average acetylcholine-equivalent content of fetal placenta was highest (0.435 gamma per gram) on the twenty-second day, at the time when the placenta begins to grow less rapidly and when it is nearly as large as it will be (Reynolds and Foster, 1939). Subsequently, a slight decrease in the acetylcholine-equivalent content of the placenta takes place. While this trend is more or less comparable to that which has been observed in the acetylcholine content of the human placenta (Haupstein, 1932), the present data are too few and too scattered to permit one to attach much significance to the higher concentration on the twenty-second day of pregnancy in the present work. It is clear, nevertheless, that a substance which resembles acetylcholine, especially in the potentiation of its action by eserine, is obtainable from the fetal placenta of the rabbit. In this

respect, therefore, the tissue from the rabbit resembles, rather than differs from, that of the human.

From the uterus, an acetylcholine-like substance was extractable at each stage of pregnancy, being present, on the average, in greatest concentration (1.363 gamma per gram of tissue) at term, least (0.279 gamma per gram of tissue) in mid-pregnancy, and intermediate in concentration during oestrus and on the twenty-second and twenty-eighth days of pregnancy. The data are too scattered, however, to attach significance to these differences, with the possible exception of the thirty-first day. In pseudopregnancy, the concentration of acetylcholine-like substance is about equal to that in the uterus in oestrus and mid-pregnancy. These relationships are shown in figure 2.

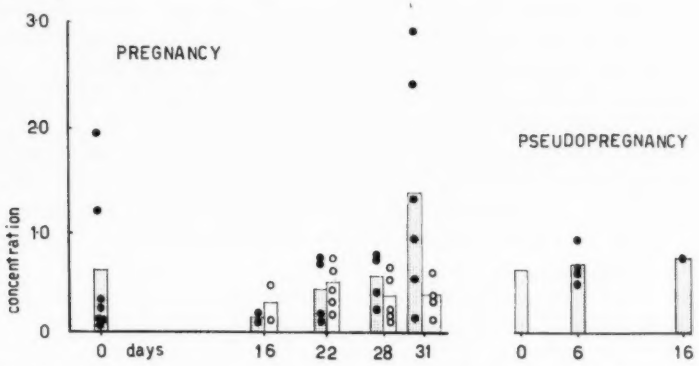

Fig. 2. Summary of data showing the concentration of acetylcholine-like substance (gamma per gram of fresh tissue) on different days of pregnancy and pseudopregnancy. The dots and circles are for individual determinations; stippled bars, average values for extracts prepared from uterine tissues; clear bars, average values for extracts prepared from fetal placental tissue.

Discussion. The significance of the presence of an acetylcholine-like substance in the placenta and uterus during pregnancy may not be stated. While there are advocates of the view that acetylcholine in the placenta at term serves as a myometrium-stimulating agent because acetylcholine administered as a drug is oxytocic, there is no conclusive evidence to show that it serves such a rôle in labor.

It is only suggestive, perhaps, that the acetylcholine-content of placental tissues from patients with uterine tetanus, premature separation of the placenta and in spontaneous abortion is higher than that found in placentas obtained from normal deliveries; in contrast, the acetylcholine content of the placenta in cases of "general uterine inertia" is low (Cattaneo, 1933). It is likewise only suggestive that acetylcholine has been used with a mea-

sure of success in slow labors, in dystocia, in uterine inertia and in placenta praevia (Bell and Playfair, 1937). In the same sense, accordingly, it is only suggestive that the acetylcholine-equivalent concentration of the rabbit uterus at term is more than twice as high, *on the average*, as at any other time in pregnancy; more than proof of its presence is required to elucidate the rôle of this substance in the uterus during labor (for discussion of the supposed rôle of acetylcholine in the uterus at term see chapter XII, Reynolds, 1939b). Direct experiments in which rabbits at term are treated with eserine, atropine, or nicotine may supply this essential proof which is now lacking.

SUMMARY

1. An acetylcholine-like substance was extracted from the fetal placenta of the rabbit on the sixteenth, the twenty-second, the twenty-eighth and the thirty-first days of pregnancy.

2. An acetylcholine-like substance was extracted from the rabbit uterus throughout the course of pregnancy. It was present on the average in lowest concentration in mid-pregnancy, intermediate in concentration on the twenty-second and twenty-eighth days of gestation and during oestrus, while on the thirty-first day it was present in more than twice the concentration (1.363 gamma per gram of uterus) found on any preceding day of pregnancy.

3. On the sixth and sixteenth days of pseudopregnancy, the concentration of an acetylcholine-like substance in the uterus was about equal to that found in the uterus during oestrus and most of pregnancy.

4. No physiological significance may be ascribed to the presence of this acetylcholine-like substance in the placenta and uterus, as has been done heretofore, from its mere presence and supposed oxytocic action. Direct proof of such a rôle in labor must be obtained by other types of experimentation.

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THE FIFTH STAGE OF TRANSMISSION IN AUTONOMIC GANGLIA

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Received for publication June 14, 1939

Rosenblueth and Luco (1939) have recently reported that upon prolonged rapid stimulation of motor nerves the period of "fatigue" (4th stage of transmission) is followed by a renewal of transmission (5th stage), as indicated by a late, persistent rise of tension.

The well-known similarity of the transmission process in neuromuscular junctions and in autonomic ganglia (Elliott, 1907; cf. Cannon and Rosenblueth, 1937) suggested that the failure of transmission during "fatigue" of autonomic ganglia might be likewise followed by a renewed ability to conduct nerve impulses through the synapse—i.e., by a 5th stage of synaptic transmission. The present study was prompted by this suggestion.

METHOD. Cats were used, anesthetized with dial (Ciba, 0.75 cc. per kgm., intraperitoneally). The changes of transmission which took place at the superior cervical ganglion when the cervical sympathetic preganglionic fibers were stimulated were followed by observations of the responses of two indicators. The first was the nictitating membrane; its contraction was recorded isotonically on a kymograph. In order to eliminate extraneous excursions of the lever due to eye movements, the eye-balls were removed, after section of the conjunctiva, the deep fascias and the extrinsic eye-muscles.

The second indicator employed was change in the diameter of the pupil. Changes were recorded either by measuring the transverse diameter with a millimeter ruler or by photographing the eye, with a constant enlargement, at different times during stimulation.

The variations of transmission in parasympathetic synapses were studied by stimulating the cardiac-slowing preganglionic fibers in one vagus in the neck and recording the corresponding changes in the heart rate. Bilateral removal of the upper thoracic sympathetic chains and bilateral section of the vagi (cephalad to the region where the stimulating electrodes were applied) were made at the beginning of the experiments to insure a denervated heart. A possible disturbing influence from variable secretion of adrenaline was eliminated by routinely tying off the adrenal glands in these experiments. The temperature of the animals was maintained

around 38° by observing a rectal thermometer and turning a heating-pad on or off as the circumstances required.

The stimuli used were condenser discharges with frequency regulated by a thyratron, controlled in turn by 60-cycle alternating current. These condenser discharges were delivered to the primary coil of a transformer and the nerves received the diphasic output of the secondary coil. The intensity of the stimuli was invariably maximal—i.e., strengthening the shocks did not lead to increase of response.

Results. A. *The 5th stage of transmission as indicated by the nictitating membrane.* Orías (1932) pointed out that stimulation of the preganglionic fibers to the membrane with a frequency of about 40 per sec. results in

Fig. 1

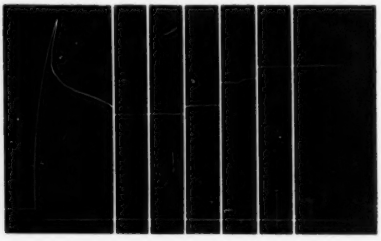

Fig. 2

Fig. 1. Influence of frequency of stimulation of the preganglionic fibers on the responses of the nictitating membrane. Lower signal: 1-minute intervals. The stimuli were applied for 2 minutes and a similar period of rest was then allowed, as shown by the upper signal. Frequencies: 1.4, 3.5, 14.5, 20, 30, 60, 95, 185 and 300 per sec., successively.

Fig. 2. The 4th and 5th stages as indicated by the nictitating membrane. Maximal continuous stimulation of the preganglionic fibers for 3 hours with a frequency of 60 per sec. The records show the beginning of stimulation, the height of the response at successive 30-min. intervals, and the end of the observation.

an initial rise of tension followed within a few minutes by a slow progressive decline; similar stimulation of the postganglionic fibers produces a better sustained response. Rosenblueth, Davis and Rempel (1936) confirmed these observations and found that the fall of the response on stimulation of preganglionic fibers took place sooner and reached a lower level as the frequency of stimulation increased up to 50 per sec. This decline of tension may be interpreted as transmission-fatigue (4th stage) in the ganglion.

In order to determine the optimum frequency for the appearance of the 4th stage observations were made covering a wider range than that which was studied by Rosenblueth, Davis and Rempel. Figure 1 is typical of the results obtained. With frequencies less than 25 per sec. the responses were sustained with little or no decline for several hours.

As the frequency increased a fairly prompt decline appeared which was maximal at about 50 per sec. Higher frequencies resulted in a less prominent decline. No frequency was found to cause a drop of tension as marked as that produced by 60 per sec. in the gastrocnemius or soleus muscle (cf. Rosenblueth and Luco, 1939).

With frequencies of from 60 to 120 per sec., if the stimulation is prolonged, the early prompt fall of the response is followed by a slower decline. About 30 minutes after the beginning of stimulation the lowest level of the response is reached. This level varies from 30 to 70 per cent

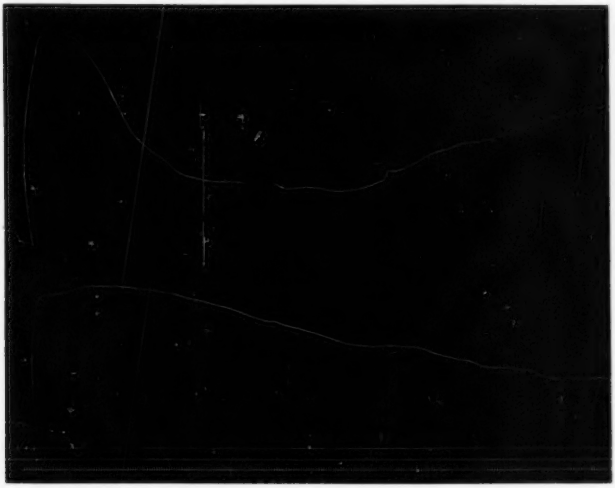

Fig. 3. Difference between preganglionic and postganglionic stimulation at 60 per sec. Right and left nictitating membranes. Upper record: preganglionic, and lower record: postganglionic stimulation. Time signal: 30-second intervals: the record covers a period of stimulation of 2 hrs.

of the original maximum. Continued stimulation leads to no further fall; on the contrary, 40 to 100 minutes after the beginning the response starts to increase and reaches a new maximum 30 to 60 minutes later. This late maximum may rise to between 50 and 85 per cent of the initial excursion. Further stimulation over a period of hours results in no significant change of the record.

In figure 2 is illustrated a typical instance. The late increase of response is similar to that encountered in skeletal muscle in analogous circumstances. It will be referred to as the 5th stage of transmission.

Stimulation of the postganglionic fibers with frequencies from 30 to 90 per sec. results only in a continuously progressing decline of the response

of the membrane. This decline is slower than that which takes place upon preganglionic stimulation—i.e., the response is better sustained (confirming Orías, 1932). No matter how much it be prolonged, postganglionic stimulation does not result in a late increase of response. Figure 3 illustrates the contrast between the two modes of activating the membrane.

If the prolonged stimulation is interrupted for brief (1 sec. to 5 min.) rest periods the response is greater upon renewal of activation than it was at the time the stimuli ceased, but it does not become as high as it was at the beginning of the experiment. These increments after rest are only transient; the record promptly drops to the level prevailing before the pause. If such a brief rest period is allowed after the 5th stage has de-

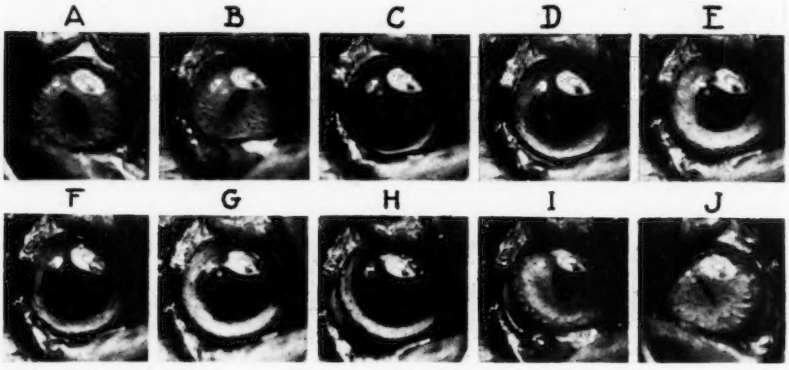

Fig. 4. The 5th stage in the iris. A and J: left pupil at the beginning and end of the experiment. B: right pupil before stimulation. C: 15 sec. after the beginning of stimulation of the right cervical sympathetic (frequency, 60 per sec.). D to H: 2, 17, 25, 30 and 45 min. after beginning of stimulation. I: 1 min. after stop of stimulation.

veloped the response upon reapplication of the stimuli drops to the high level corresponding to the 5th stage—i.e., it is better sustained than originally.

If longer periods of rest (15 to 60 min.) are given after the 5th stage has developed, it is seen that renewal of the stimuli leads to high initial responses followed by a decline to levels lower than that which corresponded to the 5th stage. The complete disappearance of the 5th stage requires from 30 to 120 min. rest. Stimulation then approximately duplicates the response recorded from the fresh system at the beginning of the experiment.

B. *The 5th stage in the superior cervical ganglion as indicated by the iris.* Stimulation of the preganglionic cervical sympathetic fibers with a fre-

quency of 60 per sec. leads to an initial maximal dilatation of the pupil. The diameter then decreases, first more rapidly, later more slowly. This decline of response is followed by a subsequent late increase, if stimulation is continued. The sequence of changes in the response is therefore similar to that described for the nictitating membrane. Figures 4 and 5 illustrate the phenomenon.

Stimulation of the postganglionic fibers at the same frequency results in maximal dilatation, decreasing only very slightly over a period of several hours.

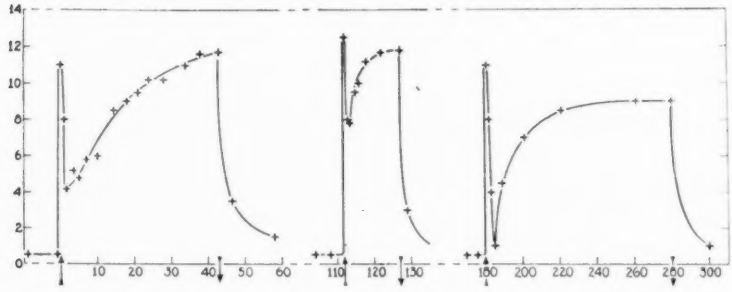

Fig. 5. The 5th stage in the iris and its subsidence with rest. Ordinates: diameter of the pupil in millimeters; abscissae: time in minutes. The arrows indicate stimulation of the cervical sympathetic with a frequency of 60 per sec.

TABLE 1

Differences in the 5th stage with the indicators used

The figures are average values. The beginning of the 5th stage is expressed in minutes after the start of stimulation. The figures in the second and third columns indicate the level of response as per cent of the maximum attained at the beginning of stimulation, which is considered as 100 per cent.

	BEGINNING OF 5TH STAGE	LEVEL OF FATIGUE	LEVEL OF 5TH STAGE
Contraction of the nictitating membrane	70	35	65
Dilatation of the pupil	15	50	100
Slowing of the heart	30	25	55

The appearance of the 5th stage was statistically more rapid in the iris than in the nictitating membrane (table 1). In 2 animals the two cervical sympathetics were stimulated simultaneously at 60 per sec.; the contraction of the nictitating membrane was recorded on one side and the diameter of the pupil was measured on the other. In both instances the 5th stage developed sooner in the iris than in the membrane.

The disappearance of the 5th stage with rest is similar in the iris to

that in the membrane as described above. Brief rest periods do not result in such a disappearance. After a rest of 30 to 60 min. the iris behaves again as originally. Figure 5 shows the progressive subsidence of the 5th stage in one experiment.

C. *The influence of the circulation in the superior cervical ganglion on the appearance of the 5th stage.* Attempts were made in several animals to record the action potentials of the postganglionic fibers during prolonged stimulation of the preganglionic trunk in order to correlate these action potentials with the corresponding 4th and 5th stages, as indicated by either

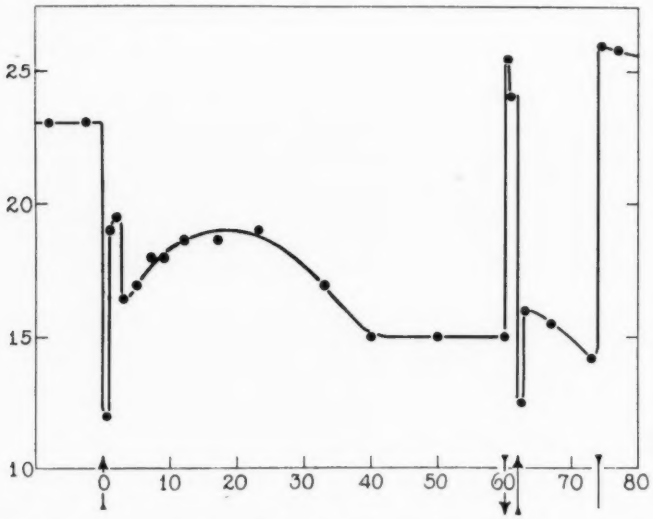

Fig. 6. The 5th stage in the cardiac slowing action of the vagus. Heart decentralized; adrenals ligated. Right vagus stimulated (as shown by the arrows) with a frequency of 60 per sec. Ordinates: heart rate per 10 sec.; abscissae: time in minutes. The rectal temperature of the animal rose from 37.8° to 38.6° in the course of the experiment.

the membrane or the pupil. For this purpose the postganglionic fibers were dissected free from the surrounding fascia and the neighboring cranial nerves and silver wire electrodes were applied to them.

No matter how carefully the dissection was made, in no instance did a late increase of response indicate the appearance of a 5th stage. Stimulation of the preganglionic fibers caused a normal initial response, promptly followed by an increasingly greater fatigue. Stimulation of the postganglionic fibers invariably resulted in normal well-sustained effects, thus showing that these fibers, and the indicator, had not been damaged by the operative procedure.

It appears likely that the disturbing factor in these experimental conditions was the unavoidable slight impairment of the circulation in the ganglion.

D. *The 5th stage in parasympathetic synapses as indicated by vagal slowing of the heart.* Peripheral stimulation of either vagus nerve in the neck (with a frequency of 60 per sec.) leads typically to the following changes in heart rate (fig. 6). The initial marked slowing is followed within 1 to 3 minutes by a rapid decline of the slowing. As a rule the rate does not attain the basal figure, but remains below normal. In one experiment, however, the heart rate was faster by 12 beats per min. 2 minutes after the beginning of stimulation of the right vagus than it had been in the basal unstimulated condition. A further slowing succeeds this relative acceleration, and this slowing then gradually subsides to reach a minimum from 20 to 50 minutes later. A late progressive increase of the response—i.e., an increasingly slower heart rate—then occurs, until 15 to 30 minutes later a fairly steady condition is attained.

It is clear that with the exception of the first acceleratory accident in the curve in figure 6, the significance of which will be discussed below, the results are the same as those illustrated in figures 2 and 5—i.e., the curves are mirror images.

The late further decrease of heart rate may be regarded as the 5th stage of transmission. A slow subsidence of this 5th stage is easily demonstrable for the heart as it is for the nictitating membrane or pupil. In figure 6 a rest period of 2 minutes left the 5th stage unabated, as shown by the persistent slowing which resulted from reapplication of the stimuli. After periods of rest of from 20 to 40 minutes, on the other hand, fatigue of the slowing action of the vagus was again demonstrable, as in the fresh preparations.

In several experiments one of the vagi was stimulated until the 5th stage had been fully reached. That stimulation was then discontinued and, without more than 1 or 2 minutes of rest, the opposite vagus was activated. The heart-rate changes yielded typical curves in which a 4th and then a 5th stage were again clearly recognizable. It is obvious that the relative persistence of the 5th stage upon stimulation of either vagus does not depend upon any modification in the heart itself, but is located within the nerve path, in all probability at the synapse in the peripheral relay from pre- to postganglionic fibers.

Discussion. The experiments of Rosenblueth and Luco (1939) showed that the 4th and 5th stages in somatic neuromuscular systems are junctional phenomena. During the 4th stage many of the nerve fibers fail to activate the corresponding muscle elements. As the 5th stage progresses more and more muscle elements share in the response because the transmission process becomes increasingly more efficient.

Although it was not possible (p. 352) in the present study to demon-

strate directly, by means of postganglionic action potentials, that during the 4th stage many preganglionic nerve impulses fail to transmit through the ganglion and that in the 5th stage renewed transmission occurs, the results strongly support the inference that such is indeed the case.

Since much better sustained responses result from postganglionic than from similar preganglionic stimulation (fig. 3, p. 349), it may be concluded with Orías (1932) that the ganglion is the site of the fatigue recorded when preganglionic fibers are excited. It is probable that the frequencies of stimulation used (mainly 60 per sec.) were sufficiently low to allow normal conduction in the corresponding preganglionic axons during the periods studied. In any event, if these frequencies should be too high for preganglionic fibers they would be even more so for the finer postganglionic elements. If failure of the axons were the cause of fatigue, postganglionic stimulation would lead, therefore, to the 4th stage sooner than preganglionic activation, and such is not the case.

Similarly with respect to the 5th stage; since it was only apparent on preganglionic stimulation, and failed to take place on exciting the postganglionic fibers, the conclusion is warranted that it denotes a synaptic phenomenon. The observations made on the effects of successive stimulation of the two vagi (p. 353) are in favor of this conclusion: when stimulation of one vagus had led to the appearance of the 5th stage, stimulation of the other promptly induced the appearance of a 4th stage—i.e., the 5th stage was not dependent on the condition of the heart.

In the observations made on the pupil (section B) the oculomotor nerve was intact. No steps were taken to eliminate its influence because this influence would presumably be adverse to the appearance of the 5th stage. The widening of the pupil elicited by sympathetic stimulation would cause an increased discharge *via* the 3rd nerve. This increased constrictor influence would oppose the late widening of the pupil during the 5th stage. Observations on the unstimulated eye showed that increased constrictor tone actually took place. On the unstimulated side the pupil became narrower when contralateral stimulation started and it remained constricted throughout the experiment (fig. 4). The absence of a 5th stage in the iris on postganglionic stimulation provides further evidence that this stage is not due to changes in the activity of oculomotor fibers.

When comparing gastrocnemius-plantaris and soleus Rosenblueth and Luco (1939) found that with a frequency of 60 per sec. the 5th stage appeared sooner in the faster (gastrocnemius) than in the slower (soleus) muscle. The difference in the rate of development of the 5th stage in the neurones distributing to the iris and in those supplying the nictitating membrane (table 1) suggests a similar relationship, since both the preganglionic and the postganglionic nerves to the iris have a lower threshold and a faster conduction velocity than those to the membrane.

The early acceleratory peak in the results of vagal stimulation (fig. 6,

p. 352) may be interpreted as follows. Fast frequencies of stimulation of motor nerve fibers produce a similar + − + sequence at the beginning of the response (1st, 2nd and 3rd phases of transmission; Rosenblueth and Luco, 1939). The trough (2nd phase) has been attributed to an excess of acetylcholine producing a depression of the muscle (cf. Rosenblueth and Morison, 1937). A similar depressing effect at the vagal peripheral relays could momentarily stop the vagal influence on the heart.

An alternative explanation might be based on the well known fact that the vagi carry a small fraction of accelerator fibers to the heart. The early changes of the response to vagal stimulation could accordingly be due to differences in the rates of establishment or in the rates of fatigue of the accelerator and the slowing influences activated simultaneously by the stimuli.

The strongest argument which the present data offer in favor of the view that there is a 5th stage of renewed transmission in autonomic synapses, succeeding the failure of transmission during fatigue, is that the sequence of changes in the response is similar in effectors as widely different as the nictitating membrane and the iris, on the one hand, and the heart rate, on the other. The only thing in common in these systems is the autonomic synapse. It is reasonable, therefore, to attribute the several stages to modified conditions at this synapse. The similarity between these nervous junctions and the somatic neuromuscular junctions is thus again emphasized by these data.

SUMMARY

The effects of prolonged rapid stimulation of the cervical sympathetic on the nictitating membrane (figs. 1, 2 and 3) and on the iris (figs. 4 and 5), and of similar stimulation of the vagus on the heart rate (fig. 6), were recorded in cats under dial anesthesia.

With all three indicators the decline of response during fatigue (4th stage of transmission) is followed by a late persistent increase (5th stage).

Postganglionic stimulation does not lead to the appearance of a 5th stage (fig. 3). It is inferred that the 4th and 5th stages are ganglionic phenomena, analogous to the 4th and 5th stages of transmission described by Rosenblueth and Luco (1939) for somatic neuromuscular junctions.

Even a slight interference with the blood supply of the superior cervical ganglion prevents the appearance of the 5th stage (p. 352).

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THE FATE OF POTASSIUM LIBERATED FROM MUSCLES DURING ACTIVITY

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Received for publication June 12, 1939

In a recent series of papers it has been shown that potassium is liberated from muscles during stimulation (Fenn et al., 1936, 1938a, b). More potassium is liberated than can be found in the blood. Experiments to be reported in this paper were designed to discover how much of the mobile potassium is in the blood and the other extracellular spaces of the body and how much is in other body cells, especially resting muscle and the liver cells.

The methods used have been adequately described in previous communications. Cats were used as experimental animals, mostly under dial anesthesia. Muscles of one or both legs were stimulated through femoral and sciatic nerves. Stimulation usually continued for 30 minutes and consisted of short tetani at a frequency of 20 to 30 per second repeated at intervals of 1 or 2 seconds. Blood, muscles and other tissues were sampled before and after stimulation and were analyzed for water and potassium and sometimes chloride and sodium.

1. *Potassium changes in the blood.* In a few experiments electrolyte analyses were made in the blood before and after stimulation. An increase of potassium concentration was found (Fenn, 1939) in arterial blood which averaged 0.1 m.eq. per 100 cc. of whole blood. This level was reached fairly promptly after stimulation began and was maintained more or less constant or decreased somewhat until stimulation ceased. Recovery was not followed. Since the loss of K from the muscle continues after the maximum concentration in the blood is reached it is evident that potassium must be leaving the blood in other parts of the body as fast as it enters the blood from the muscles.

2. *Sodium and chloride in the blood.* Malorny and Netter (1936) have analyzed blood and muscle for Na and Cl before and after stimulating hind leg muscles of cats to exhaustion. They found a greater fall of Na in the blood than could be accounted for by the rise of Na observed in the stimulated muscles. They postulated a gain of Na in resting as well as stimulated muscles. The decrease of Na in the blood was 0.9 m.eq. per

100 cc. which is about 9 times as large as the average increase of K in the blood reported above. Since stimulated muscle loses K and gains Na in approximately equivalent amounts a similar equality of change would be expected in the blood unless Na or K was removed differentially by other tissues. For better comparison it was essential to analyze blood for both Na and K after the same stimulation in one animal. Such analyses in 4 animals are given in table 1. No consistent changes in either Na or Cl are observed but there is the usual small consistent increase in K averaging 0.13 m.eq. per 100 cc. of plasma. A decrease of Na of this order of magnitude cannot be excluded by the data but a decrease of 0.9 m.eq. in whole blood or about 1.8 m.eq. per 100 cc. of plasma as reported by Malorny and Netter certainly did not occur in the somewhat less exhausting type of stimulation employed here.

TABLE 1

Electrolyte changes in blood during stimulation

(M.mols per 100 cc.)

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3		EXPERIMENT 4	
	Before	After	Before	After	Before	After	Before	After
Whole blood K	0.66	0.73	0.41	0.51	0.53	0.58		
Plasma K	0.33	0.48	0.31	0.32 (?)	0.40	0.51	0.28	0.40
Plasma K calc.*		0.46		0.46		0.48		
Plasma Na	15.3	15.1	14.6	14.7	14.1	15.0	15.1	14.8
Plasma Cl	12.5	12.7	12.1	12.4	12.4	12.0	11.8	11.7
Hematocrit, per cent	51.7	53.3	35.6	38.2	48.1	52.3	39.5	38.4

In experiments 1 and 4 all the muscles of both hind legs were stimulated; in the other two experiments, all the muscles of one hind leg.

* Plasma K calculated from hematocrit and whole blood K.

3. *Potassium increase in blood compared to the amount lost from muscles.* In a number of experiments, attempts were made to determine the total amount of potassium lost from a mass of muscles of known weight and to account for this amount quantitatively in blood and elsewhere. A number of difficulties were encountered. If only a small mass of muscles was stimulated the loss of potassium was too small to detect with certainty in the blood. If larger numbers of muscles were stimulated, many more muscle samples were required to determine the amount of potassium lost and, furthermore, fatigue came on so rapidly that contractions could not be maintained for a sufficient time to accomplish a normal potassium loss. Experiment 3 of table 1 (Nov. 1937) was the most complete technically. One leg was stimulated through sciatic and femoral nerves for 30 minutes. Blood was sampled just before and at the end of stimulation. All the stimulated muscles were removed to a large beaker and the resting muscles

to another similar beaker. After weighing, they were cut up with scissors and samples were taken for dry weight. Water content was also estimated on the remaining muscles from a determination of the specific gravity.[1] The muscles were then wet ashed and small samples were taken from the solution for potassium analyses. The samples which were dried for water content were also analyzed for potassium. It was found that the muscles lost on the average 2.6 m.mols of K per 100 grams dry weight. The stimulated muscles weighed 157 grams of which 39.8 grams was dry. Thus the total loss of K was 1.03 m.mols. The increase in the potassium content of the plasma was 0.11 m.mol per 100 cc. At this concentration the increase must have been distributed in the body in $1.03 \times 100/0.11$ or 940 cc. representing 36 per cent of the body weight. In this experiment

TABLE 2

Average volume of distribution (V_K) of potassium lost from muscles

MUSCLES STIMULATED		MUSCLE		TOTAL K LOST	PLASMA	VOLUME OF DISTRIBUTION	
Location	Dry weight	ΔH_2O cc. per 100 grams dry	ΔK m.eq./100 grams dry		ΔK	cc.	Body weight
	grams			*m.eq.*	*m.eq. per liter*		*per cent*
½ leg	8	+75 ±9	−4.9 ±0.6 (18)	0.39	0.28 (4)	1390	52
1 leg	43	+55 ±4.5	−4.2 ±0.48 (12)	1.8	1.0 ±0.14 (7)	1780	67 ±12
2 legs	86	+47 ±4.4	−1.8 ±0.65 (14)	1.5	1.5 ±0.13 (6)	1000	38 ±13

Errors represent the probable errors of the means. Average body weight = 2.67 kgm. Figures in parentheses indicate the number of differences between paired muscles or paired plasma samples which were averaged together. Eight to 10 animals were used for each group. Figures for ΔH_2O and ΔK in first group were taken from Fenn, Cobb, Manery and Bloor (1938). "½ leg" means stimulation of popliteal nerve to muscles below the knee; "1 leg" means stimulation of the whole sciatic trunk near the cord, and the femoral nerve in the groin; "2 legs" means stimulation of both femoral and both sciatic nerves.

the potassium lost from the stimulated muscles was therefore somewhat greater than that found in the chloride space or the extracellular water of the body.

A summary of all the experiments of this general type is presented in table 2. The experiments are divided into 3 groups according to the mass of muscle stimulated. The greater the mass of muscle, the smaller the

[1] Density was measured in a specific gravity bottle. The percentage dry weight can be calculated from the density of the wet muscle if the density of the dried muscle is known. These calculated values agreed closely with those directly determined if the density of the dried muscle was taken as 1.38. Unfortunately, in stimulated muscles the correct value seemed to be slightly lower, 1.35. On account of this variation direct determinations were preferred.

amount of potassium lost from unit weight of muscle and the smaller the amount of water gained. With a large mass of active muscle the supply of O_2 and foodstuffs was presumably less; possibly also it was harder to dispose of the potassium which was mobilized, for the total amount of potassium lost (and of water gained) was greater with the larger muscle masses and the increase in concentration in the plasma was also correspondingly greater. The error of these averages is relatively large especially for the Δ K in plasma when only one lower leg was stimulated, for the change is scarcely greater than the analytical error and only a few analyses were made. The same is true to a lesser degree for the Δ K in muscles of both whole legs. The ratio of the millimols of K lost from the muscles to the m.mols increase of K per liter of plasma gives the volume of distribution, V_k, or the number of cubic centimeters of plasma which would be required to account for all the potassium lost. In the last column this is expressed in per cent of the average body weight of cats, 2.67 kgm. It is found that 38 to 67 per cent of the body weight would be required to account for the potassium lost. Since the chloride space represents only about 25 per cent of the body it is probable that some potassium must have diffused into some of the tissue cells. A loss of K by the urine was eliminated either by tying off the ureters or by collecting and analyzing the urine before and after the period of stimulation. In experiment 3, quoted above, the bladder was found to contain only 0.17 m.mol of K. In one exceptional experiment the urine even before stimulation was very high in K content and enough was excreted during stimulation to account for nearly all that was lost from the muscles. Nevertheless a significant increase in K in the blood was observed. The average loss of K in the urine in these experiments certainly does not exceed 10 per cent of the total lost from the muscles.

4. *Resting muscles as depot for potassium.* It appears from these experiments that during activity of a sufficient degree of severity, potassium moves from the active muscles into inactive parts of the body, possibly resting muscles or liver. To test the rôle of inactive muscles as a depot for potassium during exercise, a number of experiments were tried in which the muscles of both hind legs were stimulated. The resting muscles of the forelegs were sampled on one side before stimulation and on the other side after stimulation, both sides being analyzed as usual for potassium and water and sometimes chloride. In another type of experiment the animal was completely eviscerated and the inflow to the liver tied off. This served to reduce the amount of tissue available for the deposition of potassium and so to accentuate the possible changes to be found in the forelegs after stimulation. In addition it was necessary to do some similar control experiments without stimulation. In each experiment it was customary to sample on both sides the following muscles; palmaris,

flexor profundus digitorum, extensor carpi radialis, triceps and biceps. Sometimes samples were also taken of kidney, skin or intestine the results of which will be referred to later.

The changes in potassium content in the foreleg muscles which can be produced by stimulation of the hind leg muscles are so small that individual experiments have no significance and it will suffice to present averages obtained from the three different types of experiments. These figures are given in table 3. In stimulation experiments with the viscera intact and in control experiments (with the viscera out) the changes of potassium are insignificant compared to the probable error of the mean. In the 5 stimulation experiments with eviscerated cats, however, involving analyses of 21 pairs of muscles, there was a gain of potassium indicated in 18 pairs of muscles, the average gain in all pairs being 0.93 ± 0.13, a clearly significant increase. The changes in Cl and water are small but, in general, the water

TABLE 3

Change in composition of resting muscles of forelegs due to stimulation of hind legs for 1 hour

Cubic centimeters H_2O and m.mols K and Cl per 100 grams dry weight

HIND LEGS	VISCERA	ΔK	P.E. OF MEAN	H_2O	Cl
Stimulated	In	+0.04	±0.18 (24)	−1	+0.02 (3)
Stimulated	Out	+0.93	±0.13 (21)	−4	−0.55 (5)
Resting	Out	−0.2	±0.33 (15)	−6	−0.55 (2)

Figures in parentheses show number of muscle pairs averaged for K (and H_2O) and for Cl. Average normal water content was 307 cc. per 100 grams dry or 75.4 per cent water. Average normal K content of foreleg muscles in m.eq. per 100 grams dry was 41.8 in palmaris longus and flexor profundus digitorum, 46.7 in the biceps and 46.7 in the triceps.

decreases when the chloride does and the ratio of chloride lost to water lost is about the same as the ratio of the amounts of these substances found in plasma. Since the control experiments were done on eviscerated cats it appears that this operation involves the loss of a small amount of tissue fluid from the muscles of the fore-legs amounting to 2 per cent of the muscle water and 9 per cent of the muscle chloride. Thus a measurable increase of potassium is found in the resting muscles only in the case of eviscerated animals; and this occurs in spite of the fact that after evisceration the muscles contract less well and the loss of potassium is presumably less than in normal cats.

The increase in potassium content of the fore-leg muscles of eviscerated cats must be due largely to penetration of potassium into the muscle cells. Assuming the largest increase of plasma potassium shown in table 2, and assuming that all the chloride space of the fore-leg muscles increases its

potassium content to the same extent as the plasma, only about 2 per cent of the increase of muscle potassium observed can be accounted for in the extracellular fluids.

Assuming that the unstimulated muscles of the body in these experiments amount to 25 per cent of the body weight it may be calculated that they would have increased in K content by about 0.9 m.mol per 100 grams dry weight if they had taken in all the 1.5 m.mols of K (see table 2) liberated on stimulation of both hind legs. This is approximately the increase found in experiments with the viscera out but with the viscera intact there is no increase within the range of the probable error of the method. This, therefore, is evidence that under normal conditions the potassium liberated in muscular activity is absorbed only to a small extent by the inactive muscles and mostly in other tissues of the body.

5. *Liver as a depot for potassium.* Having shown that more potassium can be liberated from hind-leg muscles during stimulation than can be found in the blood or chloride space of the body after stimulation, and having shown that this extra potassium cannot be found in the resting muscles, experiments were designed to discover whether, perhaps, the potassium lost from muscles was deposited in the liver possibly with the lactic acid. For this purpose the liver was analyzed for K and H_2O (sometimes also Na, Cl, N and glycogen) *a*, before and after a control period of one hour; *b*, before and again after stimulation of the hind-leg muscles for 30 to 60 minutes; *c*, before and one hour after injection of KCl. In some cases an animal used for a control experiment was subsequently used for a stimulation or injection experiment. The KCl experiments offered the advantage that the amount of mobile K was thereby accurately known and it would presumably be disposed of by the body in the same way as an equal amount of K liberated from muscles.

Methods. In sampling the liver a double ligature was drawn through the liver about 15 to 20 mm. from the edge and the 2 ligatures were tied around the edge of the liver leaving a pie-shaped segment between, which was used for analysis. The ligature apparently cut through smaller vessels but tied off the larger ones so that there was very little bleeding. Usually two or more lobes were sampled each time. A second sample was taken after injection, etc., from a comparable but not an immediately adjacent part of the same lobe. Differences between lobes appeared to be purely random and were usually not greater than the error of analysis.

In some of the stimulation experiments analyses for K were also made in the muscles and the plasma, and these results have already been reported in the average figures found in table 2. In the injection experiments the plasma was also analyzed for K and usually Cl both before and after injection. For the most part, 5 or 10 cc. of 0.1 or 0.2 M KCl were injected. From the increase of K in the plasma the volume of distribution

of the K was calculated and found to be, on the average, 70 per cent of the body weight, in agreement with Winkler and Smith (1938) and Wilde (1939). These results have been reported elsewhere (Fenn, 1939) and it was pointed out that the results might mean a concentration of K in some cells and absence from other cells rather than a uniform distribution in all the water of the body.

TABLE 4

Liver composition before and after a control period of 1 hour

NUMBER	CONDITION	POTASSIUM, M.EQ. PER KGM. WET		DRY WEIGHT, PER CENT		CHLORIDE SPACE, PER CENT	
		Before	After	Before	After	Before	After
1	D	78.2	83.9	29.4	29.4	29.0	25.1
2	d	74.4(2)	77.2(2)	29.6(2)	30.1(2)		
3	a	78.8	79.3	33.6	33.3		
4	a	80.8	85.7	31.1	31.4		
5	a	79.3	83.4	30.3	29.8	29.0	24.5
6	d	82.4	78.8	30.7	29.7		
7	d	87.2	81.8	31.3	29.1		
8	D	85.7	89.5	27.9	28.0		
9	D	87.2(2)	88.3(2)	24.6(2)	24.7(2)		
10	a	79.9(2)	77.9(2)	30.8(2)	30.4(2)	20.7	19.1
11	a	80.3	81.3	30.5	29.6		
12	u	67.8	79.3	29.2	30.3		
13	d	78.0	79.1	29.8	30.7		
14	d	65.8	78.8	27.2	28.0		
15	d	74.7	80.6	24.7	25.4		
16	d	85.7(4)	86.6(7)	29.5(2)	29.2(2)	22.2	20.1
17	d	83.2(5)	86.7(5)	28.2(2)	28.3(2)	26.3	22.4
18	d	79.5	87.0	27.5	27.3	26.3	26.0
Average		79.4	82.5	29.2	29.2	25.6	22.9

The first 7 experiments refer to the same cats as the corresponding numbers in table 7. Chloride space water is calculated as liver chloride ÷ plasma chloride × 0.96 (Donnan ratio) × 0.93 (plasma water fraction). The plasma chloride varied from 119 to 126 (av. 121) m.eq. per kgm. The control period began on the average 1 hour after the administration of the anesthetic (>1 in only 5 cats; <2 in all). D = decerebrate; a = sodium amytal 80 mgm. per kgm. intraperitoneal; d = dial of Ciba Co., 0.65 cc. per kgm. intraperitoneal; u = urethane, 10 cc. of 20 per cent by stomach tube. All figures are single determinations except where the number of figures averaged is indicated by an exponent.

a. *Control period.* The results of these experiments on 18 different cats are shown in table 4. The animals were decerebrated under ether or anesthetized with dial, amytal or urethane as indicated. The first liver sample was taken about 1 hour after the onset of anesthesia and the second one about one hour later. Plasma chloride and liver chloride were deter-

mined in 6 cats so that the chloride space of the liver could be calculated. The figures show that in each of these 6 experiments chloride space decreased, indicating a loss of extracellular fluid. The average percentage dry weight showed no change but in 15 out of 18 experiments there was an increase in the potassium per kilogram of wet weight, the average increase in all the experiments being 3.1 ± 0.5 m.eq. The probable error of the mean shows that the increase is statistically significant. The largest percentage change occurs in the chloride which must therefore be considered as primary. Since however there was no change in dry weight (the change was 0 ± 0.14 gram per 100) some dry matter must have been lost along with the extracellular fluid. A small loss of this sort would help to explain also the apparent gain in potassium content. Experimental evidence for such a loss of dry weight was found in the glycogen analyses which were carried out on 4 of these control animals. The results in table 5 show an average loss of 0.96 per cent glycogen. A similar loss under amytal and under

TABLE 5

Liver glycogen (grams per 100 grams fresh tissue) in control experiments of table 4

EXPERIMENT NUMBER	30 TO 60 MINUTES AFTER DIAL	1 HOUR LATER
	per cent	*per cent*
6	1.42	1.33
7	3.90	2.02
16	7.47	6.19
17	0.97	0.37
Average	3.44	2.48

ether anesthesia was found by Evans, Tsai and Young (1931), Tsai (1933) and Tsai and Yi (1934).

In explanation of the control experiments therefore it may be assumed that 1 kgm. of initial liver lost 11 grams of glycogen and 27 grams of extracellular fluid. If no other changes occurred the resulting liver would contain in 1 kgm. of wet tissue 82.5 m.eq. of K and 292 grams of dry matter, as observed, and $100\ (256 - 27) \div (1000 - 27 - 11)$ or 23.8 per cent chloride space as compared to the observed value of 22.9 per cent. This constitutes therefore a very reasonable explanation of the observed changes and on this basis *it is probable that no new potassium entered the liver in spite of the increase in concentration found.*

Unfortunately the observed data do not absolutely prove this conclusion. It may be assumed for example that 1 kgm. of liver lost 22 cc. of chloride space H_2O and gained 18 cc. of cell water containing as usual 176 m.eq. of K per liter. This assumption results in a K content of 82.9 compared to 82.5 in m.eq. per kgm. and a chloride space of 23.5 instead of 22.9 per cent

as found, but requires a slight increase of dry weight of 1.0 gram per kgm. Since this increase is less than the probable error of the observed difference in dry weights (0 ± 1.4 gram per kgm.) this explanation cannot be altogether excluded and it leads to the conclusion that the liver gained 3.2 m.eq. of K per kgm. of initial wet weight. Since however, this second explanation neglects the loss of glycogen observed in 4 experiments the first explanation is distinctly more probable.

TABLE 6

Liver composition before and after stimulation of muscles for 1 hour

NUMBER	TIME	POTASSIUM, M.EQ. PER KGM.		DRY WEIGHT PER CENT	
		Before	After	Before	After
1	1.3	77.8	98.0	28.3	29.3
2	0.9	71.9	73.9	30.4	30.8
3	1.5	81.8	88.0	29.9	29.7
4	1.0	78.0	88.2	31.5	31.8
5	1.9	83.4	84.4	29.2	28.8
6	2.5	82.6	89.0	33.0	32.8
7	3.0	86.0	92.1	30.6	30.8
8	1.8	87.0	86.7	32.0	32.0
9	1.7	78.5	87.2	29.3	31.0
10	ca 1.0	75.0	86.7	27.4[2]	28.4[2]
11	ca 1.3	84.4	95.4[2]	30.0	29.2[2]
12	1.8	78.8[2]	87.2[2]	30.0[2]	30.8[2]
13	1.5	80.6	86.5[2]	25.4	26.7[2]
Average	1.6	80.5	87.9	29.8	30.2

All animals were under dial anesthesia except no. 12 which was under urethane and is the same cat as no. 12 of table 4. Nos. 6 and 7 were adrenalectomized just before stimulating. The chloride space in no. 10 was 26.5 per cent before and 24.2 per cent after stimulation. Nos. 9 and 10 are the same cats as nos. 2 and 3 respectively of table 9 where values for Na and Cl are given. In no. 5 1 m.eq. of K was excreted in urine after stimulation began. In all but 4 other cats the ureters were tied or the secretion of K was negligible in amount. Time is measured from the administration of the anesthetic to the beginning of stimulation.

b. *Liver potassium during muscle stimulation.* In 13 experiments liver samples were analyzed both before and after the stimulation of the hind-leg muscles. Stimulation began between 1 and 3 hours (average 1.6 hrs.) after the onset of anesthesia, and continued for approximately 1 hour at which time the second liver sample was taken. The results are shown in table 6.

The initial potassium content is higher than in the control experiments because more time was allowed to elapse after the beginning of anesthesia and the control experiments show that potassium concentration increases

during this time. As a result of stimulation there was a slight average increase in dry weight and an increase in potassium in every case but one (where there was a negligible decrease). The changes in Na and Cl were observed in only 3 experiments the results being included as nos. 2, 3, and 4 of table 9. The last of these was not included in table 6 with the other stimulation experiments because it was performed on a cat previously used for KCl injection but the results were nevertheless entirely concordant. The results show in all cases a decrease of both Na and Cl. The chloride space may be calculated from these figures and it is found that this space decreased in the 3 experiments by 3.4, 2.3 and 2.1 cc. respectively per 100 grams wet weight, the average initial value being 27.3 per cent. This decrease in H_2O, Na and Cl presumably represents a transfer of extracellular water and salts from the liver to the stimulated muscle. In these 3 experiments the amount of water lost from 1 kgm. of liver was 14 cc. while the 3.5 m.eq. of chloride lost on the average from 1 kgm. of liver would have been contained in 29 cc. of extracellular fluid. Evidently some water presumably containing potassium entered the liver cells.

An explanation of this same type appears to fit also the average figures of table 6. Thus it may be assumed that 1 kgm. of liver lost 45 cc. of extracellular water and gained 35 cc. of cell water. In this case it may be calculated that the cell water contained K in a concentration of 80.5/(1000 − 273 − 298) or 187 m.eq. per liter. The net loss of 10 cc. of water results in an increase in dry weight from 29.8 per cent to 30.1 per cent instead of 30.2 per cent as observed. The new K content would be 87.8 instead of 87.9 m.eq. per kgm. as observed. The calculated decrease in the chloride space on this assumption would be 4.3 per cent which is probably not significantly greater than the 2.6 per cent decrease observed in (only) 3 experiments. This interpretation does not include any loss of dry glycogen as in the control experiments. This is not unreasonable since the stimulation experiments began 1.6 instead of 1 hour after the onset of anesthesia at which time, according to the work of Tsai and Yi (1934) on etherized cats, the liver glycogen is increasing instead of decreasing. Had there been a gain of glycogen it would not have been necessary to postulate so great a loss of chloride space to explain the increase in dry weight observed. These figures indicate that 1 kgm. of initial liver takes in 35 cc. of cell water containing 187 m.eq. per liter or 6.5 m.eq. In a liver of average weight, 72 grams, this amounts to 0.47 m.eq. in all.

According to table 2 the largest loss of K from the muscles during stimulation is 1.5 m.eq. Of this amount the liver is apparently able to absorb 0.47/1.5 or 31 per cent though it contains only about 3.5 per cent of the body water. If the figures for the control period be taken to indicate an intake of K of, at most, 3.2 m.eq. per kgm. without any stimulation then

the intake due to stimulation would be only 6.5–3.2 m.eq. per kgm. and only 15 per cent of the K lost from muscles would be accounted for in the liver. Even this, however, is a disproportionate amount.

c. *Liver composition after injection of potassium chloride.* In 14 cats the liver was analyzed before and one hour after the injection of large sublethal doses of potassium chloride injected intravenously. The results are shown in table 7. The weights of the animals, amounts of KCl injected and the time of the first injection after the onset of anesthesia are given as well as the amounts of potassium, of dry matter and of chloride spaces found. In this series the first analyses were made, on the average, 2.7 hours after the administration of the anesthetic and the initial K contents are correspondingly higher than in either the control or the muscle stimulation experiments. As a result of the injection the potassium increased slightly, as before, gaining 3.5 m.eq. per liter with a probable error

TABLE 7

Liver composition before and after injection of KCl

NUMBER	CONDITION	WEIGHT	M.EQ. K INJECTED	TIME	POTASSIUM, M.EQ. PER KGM.		DRY WEIGHT, PER CENT		CHLORIDE SPACE, PER CENT	
					Before	After	Before	After	Before	After
		kgm.		*hours*						
1	D	3.5	1.5	1.8	84.0	86.0	29.2(2)	29.1(2)	27.3	28.2
2	a	3.7	2.0	2.9	77.2(2)	83.5(2)	30.1(2)	30.3(2)		
3	a	2.0	1.5	2.0	80.9(2)	82.9(2)	33.5(2)	34.0(2)		
4	a	2.1	2.0	2.7	85.8	92.9	31.4	30.8	25.9	22.8
5	a	3.3	3.0	2.7	83.9(2)	80.6(2)	29.9(2)	29.7(2)	26.6	28.2
6	d	2.3	2.0	5.8	96.5(2)	102.2	28.8(2)	28.5		
7	d	2.9	2.0	1.8	81.8	89.3	29.1	29.4		
8	D	2.2	0.8	4.0	92.1	95.2	28.6	28.4		
9	a	2.8	4.0	3.7	91.0(2)	92.3(3)	28.5(2)	27.6(3)		
10	d	2.4	1.5	3.0	91.8(2)	95.9(2)	29.4(2)	28.6(2)		
11	d	2.4	2.0	3.0	86.2	85.2	32.7	30.6		
12	d	2.3	3.5	2.0	75.6(2)	87.1(2)	29.8(2)	30.0(2)	25.4	26.0
13	d	2.4	2.4	1.5	89.9(4)	88.4(4)	28.4(2)	28.6(2)	30.2	28.3
14	d	2.0	2.0	1.4	86.2(4)	90.0(4)	29.8(2)	29.2(2)	30.9	29.9
Average...		2.6	2.2	2.7	85.9	89.4	29.9	29.6	27.7	27.2

Time of injection is measured from the time of administration of the anesthetic. D = decerebrate, a = amytal, d = dial as in table 4. Nos. 1 to 7 represent a continuation of experiments 1 to 7 of table 1. The chloride space was calculated as in table 4. The plasma Cl averaged (9 expts.) 118.3 m.eq. per kgm. before and 119.5 after injection. KCl was injected in a 0.2 m solution except in experiments 1 to 4, 8, and 10 where a 0.1 m solution was used. Cats 1, 2, 6, 7, and 13 were females. Ureters were tied off in most cats, probably in all. Exponents indicate the number of figures averaged together.

of the mean of ±0.6 m.eq. Only 3 of the 14 cats showed a decrease in K. The dry weight went down 0.3 ± 0.1 per cent while the chloride space in six experiments was practically unchanged there being only a slight decrease of 5 cc. per kgm. of liver. From the average initial compositions of these livers it may be calculated that the cell water contained 203 m.eq. K per liter. It is not clear why this figure is so much larger than the 176 m.eq. per liter calculated from the control series. The figures after injection may be best explained by assuming that an initial 1 kgm. of liver lost 7 cc. of chloride space and gained 23 cc. of cellular water which was 0.203 M as to K. On this basis the new dry weight would be 29.4 per cent instead of 29.6 per cent as observed; the new potassium content would be 89.1 instead of 89.4 m.eq. per liter as observed; and the new chloride space would be 26.6 per cent instead of 27.2 as observed.

TABLE 8

Effect of injected KCl on liver glycogen and protein

EXPERIMENT NUMBER	GLYCOGEN		PROTEIN	
	Before injection	After injection	Before injection	After injection
	per cent	*per cent*	*per cent*	*per cent*
6	1.20	0.074	22.34	22.38
7	3.01	2.55	20.06	20.56
11	5.32	3.08		
13	0.40	0.29	18.69	18.53
14	5.27	5.43	18.48	18.38
Average	3.04	2.28	19.89	19.96

Numbers refer to experiments of table 7.

While all these data are satisfactorily fitted by the assumed changes there is some evidence that the injection of KCl results in a decrease of liver glycogen (cf. Silvette and Britton, 1937). Furthermore in 5 cats the liver was analyzed for glycogen before and 1 hour after the injection of KCl. The results in table 8 show that in 4 of these 5 cats there was a decrease in glycogen the average decrease in all 5 cats being 0.76 per cent. From the analyses of Fenn (1939) on rat liver it would be concluded that a loss of glycogen would be accompanied by a corresponding loss of water and potassium and chloride so that the composition of the liver as to these materials would be unchanged, while the concentrations of protein and other fixed materials would be increased. Figures for total protein of the liver (6.25 × nitrogen by Kjeldahl method) are also included in table 8 but they show no significant change. But however difficult may be the interpretation of this small glycogen loss there can be no justification for not taking it into account if it was included in the interpretation of the control experiments.

Unfortunately it becomes impossible to explain all the data satisfactorily if this glycogen loss is included unless potassium is assumed to have been taken in without water. If enough K is taken in with water, and glycogen is simultaneously lost a considerably greater decrease in dry weight should have been observed. The nearest approach to an explanation is to assume that 1 kgm. of liver lost 3 grams of dry glycogen and 10 cc. of chloride space and gained 15 cc. of K water containing 203 m.eq. K per liter. The calculation would then show a gain of 2.9 m.eq. of K instead of 3.5 ± 0.6 m.eq. K per liter as observed, a loss of dry weight of 0.4 instead of 0.3 ± 0.1 as observed, and a chloride space of 26.6 per cent instead of 27.2 per cent. This assumes however, a loss of only 0.3 per cent glycogen instead of the average 0.76 per cent which was observed although the other values do not deviate from the observed values by more than the probable error of the mean.

The amounts of potassium taken up by the liver according to these two different interpretations are 4.7 and 3.0 m.eq. per kgm. respectively. For an average liver of 72 grams weight this amounts to 0.34 and 0.22 m.eq. respectively or 15.5 per cent and 10 per cent respectively of the amount injected which averaged 2.2 m.eq. According to either interpretation, therefore, the liver has apparently taken up more than its proportionate share of the injected potassium. If the glycogen changes be neglected in both the control and the KCl experiments then the amount of KCl absorbed in the control experiments was 4.7–3.2 m.eq. per kgm. or 6.8 per cent of the amount injected, which still represents a somewhat disproportionate share.

It may be concluded tentatively that the liver has some special ability to absorb injected potassium but it must be admitted that it is impossible to prove this thesis conclusively from experiments of the type outlined. In either the KCl or the stimulation experiments, for example, it is possible that more whole liver was formed during the experiment, the calculated intake of K by the liver being increased in proportion. Conversely there may have been a decrease in the total liver in any of the experiments, thus completely invalidating the assumption made. The calculations have at least served to crystallize the difficulties involved in interpreting electrolyte analyses of liver based on wet weight only.

The results nevertheless supply some experimental evidence for the belief (Fenn, 1939) that potassium, in spite of its apparent volume of distribution in 70 per cent of the body weight is more probably concentrated in certain cells (liver) and absent from others. Although the liver may absorb a disproportionate share of any injected potassium it nevertheless receives only a relatively small fraction of the total.

6. *Sodium content of liver.* In a few experiments analyses were made for Na as well as for K, Cl, and H_2O. The results of these analyses are pre-

sented in table 9 together with some similar figures for rat livers. The sodium analyses were performed according to the gravimetric method of Butler and Tuthill (1931) as modified by Manery, Danielson and Hastings (1938), and the results were repeatedly checked on known solutions. It is surprising to find the sodium content less than the chloride content in almost every case. In rabbit livers Manery, Danielson and Hastings (1938) found the Na/Cl ratio the same as in an ultrafiltrate of plasma. The reason for this discrepancy is not apparent. It cannot be due to varying amounts of blood in the liver for the Na/Cl ratio is unusually high in cat whole blood and the amount of blood in the liver is never greater

TABLE 9

Electrolytes in liver (per kgm. fresh tissue)

NUMBER	CATS	K	Na^+	Cl	H_2O	PLASMA Cl
		mM	*mM*	*mM*	*cc.*	*mM*
1	Initial	78.2	30.5	40.8	706	126
	One hour later	83.9	32.6	35.3	706	123
2	Initial	78.5	34.0	38.4	707	
	After muscle stim.	87.2	31.5	33.7	690	
3	Initial	75.0	33.3	35.6	736	
	After muscle stim.	86.7	33.2	32.5	714	
4	Initial	85.7	33.1	33.2	686	116
	After KCl injection	92.8	28.8	27.2	692	
	After muscle stim.	95.6	22.8	24.4	689	
	RATS					
5	Initial	97.4	26.1	28.4	702	106
6	Initial	94.6	24.7	28.1	689	109
7	Initial	102.0	27.7	30.9	709	100
8	Initial	93.7	20.8	30.0	704	98

Nos. 2 and 3 are nos. 9 and 10 respectively, of table 6; nos. 1 and 4 are nos. 1 and 4 respectively of tables 4 and 7.

than 5 per cent after excision and blotting. The variations due to control period, KCl and muscle stimulation are in general similar to the changes already described, there being in all cases a rise in potassium and a fall in chloride and sodium. The water content goes down after muscle stimulation, goes up after KCl injection, and shows no change after the control period.

Since there is no sodium in liver in excess of the chloride and since the chloride is in all probability (Truax, 1939) entirely extracellular it appears that the sodium also must be extracellular or in a different part of the liver from the majority of the potassium. For this reason it is difficult to explain an increase of potassium in the liver as an exchange for an equivalent amount of sodium.

7. *Movements of water in the body as a whole during stimulation.* In a number of experiments samples of kidney, skin and intestines (as well as resting fore-leg muscles, table 3) were taken before and after stimulation of the hind-leg muscles and were analyzed for water and in some cases for electrolytes. The results are summarized in table 10. Values for liver and resting and stimulated muscles from other tables in this paper are also included for comparison. The water is expressed in cubic centimeters per 100 grams of dry tissue. It can be seen that all these, with the exception of resting muscles, lost significantly greater amounts of water during stimulation of the hind-leg muscles than in similar control experiments without stimulation. While the control experiments are not perhaps quite adequate to prove that no change would have occurred in the absence of stimulation it appears probable that the muscles on stimulation

TABLE 10

Loss of water from tissues during stimulation of muscles

TISSUES	CONTROL CATS		STIMULATED CATS		NORMAL DRY WEIGHT	WEIGHT PER CENT BODY WEIGHT	WEIGHT DRY	Δ H_2O
	Number of analyses	Δ H_2O, cc. per 100 grams dry	Number of analyses	Δ H_2O, cc. per 100 grams dry				
					per cent		*grams*	*cc.*
Kidney	2	−7	4	−30	22.0	0.81	4	−1
Skin	3	+9	7	−19	30.4	13.9	97	−18
Intestine	2	0	6	−13	20.4	3.8	18	−2
Liver	18	0	14	−4	29.2	2.96	20	−1
Resting muscles			24	−1	25.0	35.7	205	−2
Stimulated muscles			10	+47	25.0	9.6	55	+26

Weight of tissues in per cent of body weight largely from Skelton, 1927. Percentage dry weights are averages from author's analyses. Data for stimulated muscles from table 2, both legs being stimulated.

increase their water content at the expense of non-muscular parts of the body.

In order to follow the movements of water in more detail the total amount of dry matter in each of the tissues was calculated in table 10 from the average percentage dry weight of each of the tissues and the weight of the tissue in per cent of the body weight (from Skelton, 1927) assuming an average body weight of 2.3 kgm. Knowing the Δ H_2O per 100 grams dry it was possible to calculate the cubic centimeters H_2O transported to the muscles from each of the tissues concerned. These figures are given in the last column of table 10. They show that on stimulation of both hind legs 26 cc. were taken up by the stimulated muscles of which 24 cc. were accounted for in the tissues named and of which 18 cc. came from the skin and only 1 cc. from the liver. Along with this loss of H_2O the skin pre-

sumably lost NaCl and in one experiment losses of 10 and 15 m.eq. of Na and Cl respectively per 100 grams dry weight were actually observed.

These figures must be regarded as only approximate estimates on account of the relatively small number of cats used. This is particularly true of the intestines where 3 of the 6 samples actually gained in water content. This extreme variability probably depended on the technique of preparing the intestine which was cut open, scraped free of mucosa and blotted.

Discussion. The experiments presented appear to indicate that muscular activity liberates potassium which is taken up in part by the liver. In recovery the reverse changes must occur since it has been shown that the muscle regains the potassium which has been lost. This potassium cycle is comparable, therefore, to the well known carbohydrate cycle and it is possible that the potassium is transported as potassium lactate.

While the evidence certainly points to the existence of such a cycle it would be a mistake to overemphasize it without further proof. A large number of observations have been necessary in order to obtain a statistically significant result and individual experiments do not always fit into the expected picture. The probable error of the means is such that the deposition of some potassium in the resting muscles or other parts of the body than the liver cannot be excluded. Several pieces of work have shown that when the arterial blood is high in potassium the venous blood from muscles is lower, indicating the deposition of potassium in resting muscles (Mond and Netter, 1930, in frogs; Houssay and Marenzi, 1937, in dogs). In perfused frog muscles this result has been frequently confirmed by the author. When potassium is injected intravenously in large amounts Houssay and Marenzi (1937) have also observed an increase in the concentration of potassium in the muscles per unit wet weight by direct analysis of the muscles. The author's occasional observations on this point with rather smaller injections have been inconsistent and in stimulation experiments no significant increase was found except in eviscerated animals. It must be remembered, however, that the amounts of potassium concerned both in injection and in stimulation are very small compared to the large amounts normally present and the exact whereabouts of such mobile potassium is practically impossible to discover with absolute certainty by the methods used in this work. Since some four times as many experiments would be necessary to double the accuracy of the results it has become obvious that some other method of attack is necessary for a final proof. It may be said however, that the present data make it highly probable that the liver does absorb more than its share of mobile potassium especially when that potassium is liberated from stimulated muscles. Even in that case, however, only about $\frac{1}{3}$ of the mobile potassium is found in the liver. The evidence further indicates that such

potassium as is taken up by the liver enters in the form of an approximately isotonic solution of potassium comparable in composition to the cell water. To keep the osmotic pressure constant this would be necessary unless K exchanged for Na. The absorption of potassium by the liver is apparently confirmed by Houssay and Marenzi (1937) who found an increase in wet weight potassium in the liver after large injections. Without simultaneous measurements of water and chloride, however, this result proves nothing with certainty. The difficulty of locating potassium by wet weight analyses only have been amply shown by the detailed consideration of the data of tables 4, 6 and 7. Further confirmation of the absorption of potassium by the liver, however, has been supplied by Zwemer and Pike (1938) who have shown that the hepatic vein blood contains less potassium than the portal vein blood when the potassium level is raised by splanchnic stimulation. It might also be mentioned that Greenberg, Joseph, Cohn and Tufts (1938) have found that artificially radio-active potassium is taken up by the liver but not in amounts greater than by muscle, kidney, and heart. This result, however, tells merely how readily injected potassium can exchange with potassium previously present and tells nothing about the location of the excess potassium.

As evidence against the proposed potassium cycle the work of Kaunitz and Selzer (1938) should be mentioned. These authors report that in rats exercising in a treadmill for 24 hours there is a loss of 2 m.mols of potassium and a gain of 0.8 m.mol of sodium in 100 grams of wet liver. In these experiments however, the exercised animals were compared with resting control animals starved for an equal length of time so that other factors of general bodily fatigue may have been introduced. Furthermore, the variations in normal potassium content quoted are so large (from 165 to 565 mgm. per cent) that some disturbing factors must be suspected.

SUMMARY

An attempt was made to discover the fate of potassium injected into the blood stream or liberated from muscles of cats by motor stimulation. In both cases the increase in potassium concentration found after 1 hour in the blood is such as to indicate that more than half of the potassium must have penetrated into some cells of the body and is not located in the interstitial or circulating fluids. Analyses for potassium, water and chloride in resting muscles show that they do not absorb detectable amounts of potassium from stimulated muscles. Similar analyses in liver show that this organ probably takes up 15 per cent of injected potassium and 31 per cent of potassium liberated from active muscles although it accounts for less than 3 per cent of the body water. Moreover, it appears that this potassium enters the liver cells in the form of an approximately isotonic solution. To some extent this gain of cell water is accompanied by a

corresponding loss of extracellular fluid from the liver containing chloride but most of this Na, Cl, and H_2O taken up by the stimulated muscles comes from the skin.

I am indebted to Mrs. Doris Cobb Marsh and Miss Eugenia Sheridan for most of the chemical analyses reported in this work.

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THE EFFECT OF ABLATIONS OF NEOCORTEX ON MATING, MATERNAL BEHAVIOR AND THE PRODUCTION OF PSEUDOPREGNANCY IN THE FEMALE RAT AND ON COPULATORY ACTIVITY IN THE MALE[1]

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Received for publication July 12, 1939

The work of Beach (1937) demonstrated that destruction of large portions of the cerebral cortex of female rats resulted in some impairment of their maternal behavior patterns. Although lesions inactivating ten per cent of the cortex produced detectable deficiencies of response in certain of the tests made, marked deficiencies were observed only in those animals which lacked more than twenty per cent of the cerebral cortex. Stone (1939) found that destruction of twenty-five per cent of the cortex failed to modify copulatory activity, gestation, parturition or the maternal behavior of rats. Inactivation of more than 65 to 80 per cent of the cortex did not affect mating, gestation and parturition but did abolish most of the maternal behavior. Stone also found that removal of 50 per cent of the cerebral cortex from immature male rats did not prevent the development of normal mating reactions. One purpose of the experiments to be reported in this paper was to ascertain the effects of complete ablation of the neocortex on mating and the reproductive behavior of adult male and female rats.

The phenomenon of pseudopregnancy was also studied in a series of animals before and after partial and complete ablation of the neocortex. Long and Evans (1922) found that female rats which are mated with vasectomized males show many changes characteristic of the early stages of pregnancy. The estrous cycles cease for twelve to sixteen days; corpora lutea persist; progestational changes occur in the uterus and the mammary glands develop to some degree. This condition has been called pseudopregnancy. There is evidence which indicates that a neural mechanism is involved in the initiation of pseudopregnancy in the rat (Meyer, Leonard and Hisaw, 1929). The phenomenon can be produced by sterile mating, mechanical and electrical stimulation of the cervix and vagina (Long and

[1] This work was aided by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.

Evans, 1922; Slonaker, 1929; Shelesnyak, 1931), by direct electrical stimulation of the brain (Harris, 1937) and in numerous other ways (Selye and McKeown, 1934; Shelesnyak and Rosen, 1938; Weichert and Boyd, 1933; Wolfe, 1935). Vogt (1931), Haterius (1933), Friedgood and Bevin (1938) and others have shown that mating is more effective than electrical and mechanical stimuli in the production of pseudopregnancy. Meyer, Leonard and Hisaw (1929) found that mechanical and electrical stimuli are even less effective in anesthetized animals. These observations have led to the suggestion that sexual excitement is a causative factor in the production of pseudopregnancy. It has been shown to be the factor which determines ovulation in the rabbit (Brooks, 1937). The observation of the relationship between the amount of stimulation and the percentage of success in the elicitation of pseudopregnancy also suggests that a discriminating mechanism is involved (Ball, 1934). On the basis of this work it seemed reasonable to conclude that ablation of the cerebral cortex might at least modify the effectiveness of various pseudopregnancy-producing stimuli. The validity of this hypothesis was tested by a study of the pseudopregnancy response of rats before and after partial or complete removal of the neocortex.

METHODS. The female rats used in this work were large animals which showed normal estrous cycles. Both males and females were decorticated in two stages, two weeks to a month elapsing between operations. The cortical tissue was removed with an aspirator and very little subcortical injury resulted. Following each operation there was a short period of anestrus which lasted until the animal regained its normal weight and vigor. Vaginal smears were taken daily with a pipette and a small amount of 0.9 per cent saline. Six of the brains were eventually sectioned serially, stained with gallocyanin and the extent of the lesions determined histologically. The brains of the remaining animals were preserved and a careful macroscopic examination of the lesions was made.

In the study of pseudopregnancy the effects of coitus with vasectomized males and of stimulation of the cervix and vagina with glass rod or electrical current were tested. Electrical stimuli were applied by means of a small concentric electrode fused into a glass rod. The stimulator employed delivered a 60-cycle sinusoidal wave. During stimulation a current flow of 0.2 milliampere was maintained. The electrode-rod was moved continuously and various points on the cervix and vaginal wall must therefore have been stimulated. This constituted a mixture of electrical and mechanical stimulation. The stimulation was applied for three two-minute periods with one minute of rest intervening. When pure mechanical stimuli were used a small rod 3 mm. in diameter was inserted in the vagina and moved in and out approximately 200 times within one minute. This was repeated after an interval of 15 minutes. These tests

were made first on the animals before operation, again following hemidecortication and finally after removal of the neocortex and a large part of the pyriform lobe. In addition to observing the delay in onset of a subsequent estrual period decidual reactions were used in many instances as criteria of the occurrance of true pseudopregnancy. A thread was passed through a segment of the uterus on the third to fifth day following mating or stimulation. That portion of the uterus was removed on the 8th–12th day, fixed, sectioned and studied histologically.

A series of hemidecorticate and fully decorticate female rats were mated with normal males. These females were observed during gestation and immediately after parturition and their maternal behavior studied. Some animals were kept for nine months after complete ablation of the neocortex.

A group of adult male rats were similarly prepared. Before any male was subjected to a cortical ablation its mating responses were tested by isolating it with one or two normal females. The presence of sperm in the vaginal smear was used as proof that intromission had occurred. Mating tests were made following hemidecortication and again after complete removal of the neocortex. If mating failed to occur following these operations, testosterone, 5 mgm. every second day, was injected intramuscularly over a period of a week or ten days. Several of these males were kept for five months following the final cortical ablation.

Results. *Females.* Mating, gestation and parturition appeared to be perfectly normal following hemidecortication and even after removal of most of the neocortex. No effort was made, however, to measure the frequency of mating, the normality of the mating and parturitional activities or the ratio of frequency of mating to impregnation. Three hemidecorticate rats successfully reared very large litters. Two of these had previously had young and the maternal behavior before and after hemidecortication was similar. Four hemidecorticate rats for some reason did not care for their young normally. Some of the control females which had not been operated upon also showed deficient maternal behavior. Only one of these 4 hemidecorticate rats had previously been pregnant and reared young. Obviously abnormalities and deficiencies other than those produced by cortical lesions occur which can affect maternal behavior. All of these animals which had failed to show normal reactions were discarded. It is felt that the three cases in which maternal behavior was normal adequately demonstrate that hemidecortication does not affect this type of reaction. The maternal behavior of all of the bilaterally decorticate rats was affected. Sixteen females from which all or nearly all neocortex had been removed bilaterally were placed with normal males. Four of these females had reared litters before operation and had shown normal maternal behavior. All of the sixteen animals mated but only twelve became pregnant and only eight came to term. Among this group

were 4 from which all neocortex had been removed. None of the eight displayed any of the reactions typical of maternal behavior. They failed to nurse, clean or retrieve their young. There were no indications of nest building even though nesting material was available. The young were normal and when given to foster mothers lived and reached maturity.

The estrous cycles of rats which had undergone unilateral removal of the neocortex reappeared within a week or twelve days following operation. These cycles were entirely normal, averaging from 4 to 5 days in duration. The sensitivity of the hemidecorticate animals to stimuli which normally produce pseudopregnancy was determined. The results of these experiments are summarized in table 1. Even in the control animals the per-

TABLE 1

Summary of the results obtained with various pseudopregnancy-producing stimuli before and after ablation of the neocortex

METHOD OF STIMULATION	NUMBER OF ANIMALS	TRIALS	CASES OF PSEUDO-PREGNANCY PRODUCED	PER CENT SUCCESS
Control rats (22 animals)				
Glass rod	10	28	17	61
Sterile mating	17	32	21	66
Combined electrical and mechanical	17	28	19	67
After unilateral ablation of the neocortex (14 animals)				
Glass rod	9	25	8	32
Sterile mating	12	30	15	50
Combined electrical and mechanical	9	18	12	66
After bilateral ablation of the neocortex (16 animals)				
Glass rod	9	24	7	29
Sterile mating	9	25	12	48
Combined electrical and mechanical	9	22	14	64

centages of successful stimulation were not in very close agreement with the percentages obtained by Haterius (1933) or Vogt (1931, 1933) but the type and duration of stimulation also differed. Electrical and mechanical stimuli which proved to be most effective under the existing conditions were employed.

Sixteen hemidecorticate rats survived ablation of the neocortex. In fourteen, normal estrous cycles reappeared within two weeks after completion of the cortical ablation. The other two females never developed regular cycles although they occasionally had a normal estrous period. Table 1 summarizes the results obtained when these bilaterally decorticate rats were subjected to sterile mating, glass rod and electrical stimulation

of the cervix and vagina. Figure 1 is a typical record of the history of one animal. It shows how the estrous cycle was affected by the operations and various stimuli.

The duration of pseudopregnancy was approximately the same in normal, hemidecorticate and bilaterally decorticate rats. In certain individuals of each group the ability of the uterus to give a decidual reaction during pseudopregnancy was tested and in all cases histological studies showed that a positive reaction had occurred. These experiments demonstrate that the neural pathways and integrating centers essential to the pseudopregnancy response are still intact after unilateral and bilateral ablation of the neocortex. It was observed, however, that hemidecorticate rats were less responsive to the pseudopregnancy-producing stimuli than were normal females. In the bilaterally decorticate rats pseudopregnancy was least easily produced. Electrical stimuli, however, appeared to be equally effective in the three groups of animals.

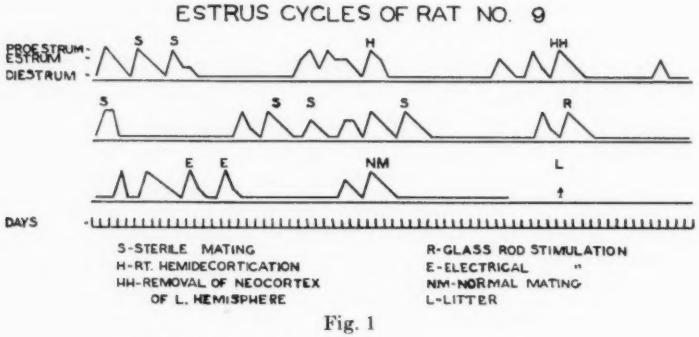

Fig. 1

Males. Unilateral removal of the neocortex did not abolish mating activity in any of the male rats. Following bilateral ablation of this neocortex 4 male rats failed to mate even after a series of testosterone injections was given. Four males continued to mate after bilateral ablation of the neocortex and 3 more mated when given testosterone. The brain lesions of the animals which mated and of those which failed to mate following bilateral removal of the neocortex were grossly similar. An examination of sections of the brains of two animals from each group failed to reveal any obvious differences.

Brooks (1937) found that male rabbits continued to mate following bilateral ablation of the neocortex but subsequent removal of the olfactory bulbs abolished this activity. The olfactory bulbs were removed from one male rat which had continued to mate following the bilateral ablations of neocortex. The animal continued to mate but at autopsy it was found that remnants of the neocortex of both hemispheres remained.

DISCUSSION. Although it has been suggested that pseudopregnancy in the rat may be the result of an emotional or nervous excitation of the hypophysis the fact that the phenomenon can be produced in such a variety of ways indicates that it is not a very specific reaction. At any rate the neocortex is not essential to the response though the central nervous system is undoubtedly involved. Sensory deficiencies resulting from decortication might conceivably explain why mating and glass rod stimuli are less effective following ablation of cortical tissue. Our results show, however, that combined electrical and mechanical stimulation is practically as effective following decortication as before.

These experimental results agree with those of Beach (1937) and Stone (1939) in showing that the neocortex is not essential to mating responses in the female. The male also continues to mate following ablation of nearly the entire neocortex of both hemispheres. Although no accurate measurements were made of the incidence of mating or of the sex drive of the bilaterally decorticate females they appeared to be less active and to mate less readily and frequently. The fact that some males did not mate until testosterone injections were given tends to support this conclusion. All of the bilaterally decorticate females mated but again no statement can be made concerning the comparative frequency of mating. The more complex activities of the female such as nest building, cleaning and retrieving of the young are abolished by ablation of neocortex. It can therefore be stated that the neocortex is essential to the maternal behavior of the rat.

SUMMARY

Pseudopregnancy can be elicited in the rat following bilateral ablation of the neocortex. The duration of pseudopregnancy is the same as in normal rats but the phenomenon is somewhat more difficult to obtain in the decorticate animal.

After bilateral removal of the neocortex rats still have normal estrous cycles, mate, become pregnant and give birth to normal young but they show no maternal behavior.

Ablation of the neocortex does not necessarily abolish mating behavior in the male although a few animals among those studied could not be induced to mate after their neocortex had been removed.

This problem was suggested by Dr. Chandler McC. Brooks and I wish to thank him for the assistance received.

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THE FORMATION OF ACETYLCHOLINE BY TISSUES OF THE RAT[1]

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Received for publication July 8, 1939

Quastel, Tennenbaum and Wheatley (1) have demonstrated an *in vitro* production of acetylcholine by various tissues and elucidated the factors influencing this production. It was observed that brain cortex slices under aerobic conditions in the presence of glucose produced amounts of acetylcholine far in excess of the other tissues studied. Stedman and Stedman (2) have confirmed the production of acetylcholine-like substance by brain tissue. They have demonstrated its presence not only by its action on eserinized leech muscle but also by actual isolation as the double chloroplatinate of choline and acetylcholine. The present investigation is concerned with: 1. The influence of quinine on acetylcholine formation. 2. The amount of acetylcholine produced by infant as compared to adult cerebral tissue. 3. The rate of formation of acetylcholine by brain, intestine, and mucosa. 4. The effect of glucose on acetylcholine production by cerebral tissue. 5. The preformation of the substance in tissues.

METHOD. The methods used are similar to those described by Quastel et al. (1). The brains of both adult and newly born rats were sliced and placed in the Ringer-phosphate-eserine solution for various periods of time. Small volumes of this fluid medium were added to the solution in which the leech strip was suspended. The resulting muscular contractions were measured on a smoked drum. In other experiments, whole intestine and separated smooth muscle and mucosa were also used. No attempts were made to calculate quantitatively the amount of ester formation. Instead, paired contractions were recorded with each leech strip and four strips used with each experiment. No results with any strip of leech muscle were accepted unless confirmed on the other strips.

RESULTS. Each of the tables below presents results obtained on only one of four strips studied simultaneously. Table 1 contains typical results of the influence of quinine on acetylcholine formation as observed in 6 experiments with adult rat brain, 3 with whole intestine, 3 with intestinal smooth muscle, and 3 others with intestinal mucosa. In all cases quinine increases the formation of acetylcholine.

[1] Aided by a grant from the Child Neurology Research (Friedsam Foundation).

Studies on the rate of acetylcholine formation are presented in table 2. In experiment I, it is seen that in two succeeding five minute periods similar amounts of acetylcholine are produced by the same rat brain sample. However, in experiment II a comparison between the quantity of acetylcholine produced in 5 minutes with that of a period of 60 minutes reveals that although in the latter the amount of acetylcholine is larger, the rate of production diminishes significantly with time. Experiments III on infant brain and IV and V on intestinal muscle and mucosa respectively reveal similar results. As seen in experiment VI the presence of glucose appears to have no effect on the rate of acetylcholine production.

In 12 experiments, a comparison of acetylcholine production of infant and adult cerebral tissue per 100 mgm. moist weight reveals (table 3) a larger production of acetylcholine by the infant tissue. This difference would be increased still more if the acetylcholine production were calculated

TABLE 1

Effect of quinine on acetylcholine formation

(1 cc. of medium, 2 mgm. of quinine)

EXPERIMENT NUMBER	60 MINUTES	HEIGHT OF CONTRACTION	FLUID MEDIUM
		mm.	*cc.*
1	Non-quinine adult rat brain	41.0	0.4
	Quinine adult rat brain	91.0	0.4
2	Non-quinine whole intestine	76.0	0.2
	Quinine whole intestine	105.0	0.2
3	Non-quinine intestinal smooth muscle	78.0	0.1
	Quinine intestinal smooth muscle	96.0	0.1
4	Non-quinine intestinal mucosa	33.0	0.5
	Quinine intestinal mucosa	46.0	0.5

on a dry weight basis because the dry weight of younger tissues is proportionally less than that of adult ones.

Discussion. Harvey (3) has already elucidated the mechanism of quinine action in myotonia congenita. This drug produces a curare-like effect on the myoneural junction which decreases the motor response to a series of stimuli. Despite this action he also noted an increased response to a single maximal stimulus. The present results reveal a possible reason for this increased response to a single stimulus, namely, a greater production of acetylcholine-like substance after the addition of quinine. It is interesting that not only striated muscle but also smooth muscle, as well as brain, all produce increased amounts of acetylcholine in the presence of quinine. Perhaps the stimulating action of small doses of quinine may be attributed to the augmented production of acetylcholine.

In an attempt to study the rate of formation of acetylcholine, experi-

ments were performed in which the tissue was bathed for successive five minute periods in the fluid medium, which was then decanted and assayed for acetylcholine. A comparison between the first and second decantations revealed that in both cases a similar amount of acetylcholine was formed. This suggests that acetylcholine is rapidly formed and indicates the existence of a ready precursor for this formation. Furthermore, the rate of production is seen to be more rapid in the 5 minute period than subsequently (60 min.). In the various tissue studies, intestine, adult brain,

TABLE 2

Factors influencing production of acetylcholine

EXPERIMENT NUMBER	SAMPLES	HEIGHT OF CONTRACTION	FLUID MEDIUM
		mm.	*cc.*
1	First 5 minute decantation adult rat brain	38.0	0.2
	Second 5 minute decantation adult rat brain	41.0	0.2
2	5 minute adult rat brain	11.0	0.3
	60 minute adult rat brain	27.0	0.3
3	5 minute infant rat brain	21.0	0.3
	60 minute infant rat brain	34.0	0.3
4	5 minute intestinal smooth muscle	30.0	0.5
	60 minute intestinal smooth muscle	62.0	0.5
5	5 minute intestinal mucosa	58.0	0.4
	60 minute intestinal mucosa	95.0	0.4
6	60 minute non-glucose adult brain	42.0	0.2
	60 minute glucose adult brain	39.5	0.2

TABLE 3

A comparative study of acetylcholine production of infant and adult rat brain

Infant, 60 minutes, dry weight = 0.0140 gram. Adult, 60 minutes, dry weight = 0.0186 gram.

LEECH STRIP A	HEIGHT OF CONTRACTION	FLUID MEDIUM
	mm.	*cc.*
60 minute infant rat	91.0	0.4
60 minute mature rat	35.0	0.4

and infant brain more than $\frac{1}{3}$ the amount of acetylcholine formed in one hour was present in five minutes.

It is interesting to note that the brain of infant rats produces a greater quantity of acetylcholine than does the cerebral tissue of adult rats, despite the fact that per gram of dry weight a much smaller amount of infant tissue was employed. Nachmansohn (4) has observed greater activity of choline esterase in adult than in the newly born rats. It is, therefore, possible that the greater amount of acetylcholine present in the

young may be attributed to the lower choline esterase activity of these tissues. We were able to confirm our earlier finding (5) that glucose does not increase the rate of production of acetylcholine by cerebral tissue (2). In view of the fact that the part played by acetylcholine in the central nervous system is under active investigation, we shall not discuss the significance of these findings in respect to the function of the central nervous system.

CONCLUSIONS

In the present investigation the production of acetylcholine by the brain of infant and adult rats and intestinal smooth muscle and mucosa of mature rats was measured by its effect on eserinized leech muscle. It was observed that:

1. Quinine increases acetylcholine production in all tissues studied.
2. There is a ready precursor for the formation of acetylcholine.
3. Acetylcholine is formed rapidly, with a large formation in first 5 minutes.
4. The rate of formation is not influenced by the presence of glucose.
5. There is a greater production of acetylcholine in the brain of the new-born than in that of adult rat.

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THE ELECTROLYTES OF MUSCLE AND LIVER IN POTASSIUM-DEPLETED RATS

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Received for publication July 3, 1939

The gross effects of withholding potassium from the diet of young rats has been the subject of a number of investigations (Osborne and Mendel, 1918; Miller, 1923; Leulier and Vanhems, 1934; Schrader, Prickett and Salmon, 1937; Grijns, 1938; Heppel and Schmidt, 1938). In general it was found that the deficiency results in failure to continue growth followed by death after a variable number of weeks. No reports are available in the literature dealing with chemical studies of the tissues of potassium-depleted animals. The present investigation was designed to determine the effect of potassium deprivation on the concentration of the principal electrolytes in serum, muscle and liver tissue.

In normal muscles the concentration of potassium is much greater than that of sodium. It is generally held that potassium is concentrated in the intracellular phase while sodium is largely restricted to the extracellular phase (see Fenn, 1936, and Manery and Hastings 1939). The most striking result of the work reported here lies in the fact that the muscles of animals deprived of potassium take up sodium instead of potassium. This occurs to such an extent that in some instances the muscles of the experimental animals are found to be richer in sodium than in potassium. We have here a clear-cut example of a condition in which sodium must occur largely as an intracellular cation.

EXPERIMENTAL. The composition of the diets is given in the footnote.[1] The rice-bran extract and liver extract were treated to remove potassium according to the procedure used by Miller (1923). This involved the precipitation of potassium bitartrate from an alcoholic solution of the extract. After removal of the precipitate, the filtrate was concentrated

[1] The experimental diet contained 50.9 per cent of sugar, 10 per cent of vegetable fat, 25 per cent of acid-washed casein, 4.1 per cent of salt mixture, 2.0 per cent of Lilly's liver extract (reëxtracted with 6000 cc. of 70 per cent ethanol per kilo of extract), 3.0 per cent (cc. per 100 grams of diet) of Vitab rice-bran extract (reëxtracted with 3700 cc. of 75 per cent ethanol per liter of extract), 1 per cent of wheat germ oil and 1.5 per cent of cod liver oil. The composition of the salt mixture (modified Steenbock 32) was as follows (grams): $CaHPO_4 \cdot 2\ H_2O$, 223; NaCl, 40; $MgSO_4 \cdot 7\ H_2O$, 127; Na_2HPO_4, 41; $Ca(C_3H_5O_3)_2 \cdot 5\ H_2O$, 57; $Fe(C_6H_5O_7)$, 27; NaI, 1.8; $CaCO_3$, 199.

by means of vacuum distillation. The potassium-low diet contained 0.01 per cent of this element.

Young albino rats from the stock colony were transferred to the experimental ration when they reached a weight of 90 to 100 grams. The potassium-deprived rats were maintained on the diet for a period of approximately 45 days. In most cases there was no change in weight during this time. Some of the animals, however, gained up to 20 grams while a few others lost up to 15 grams. No external abnormalities became evident except for a little roughening of their fur. Studies of the gross and histological changes in the tissues are now being carried out (see Schrader, Prickett and Salmon, 1937).

A group of control animals were maintained on the same basal diet supplemented with potassium hydrogen phosphate, so that it contained 0.55 per cent of potassium. These rats gained from 20 to 30 grams in weight per week. (Growth curves have been published by Heppel and Schmidt (1938).) They were sacrificed after subsisting on the ration for 35 days.

The third group of animals whose tissues were analyzed consisted of young rats from the stock colony weighing approximately 90 grams.

At the end of the experimental period the rats were pooled for analysis into groups of 2 to 3 animals, as indicated in the first column of tables 1 and 2. At first the blood was collected without exposure to air while the animals were under urethane anesthesia, as described by Smith and Smith (1934). This method was used in the case of the first four groups of rats listed in table 1. The deficiency seemed to render the animals particularly sensitive to anesthetics and it was difficult to adjust the dosage. For this reason, all of the other groups of rats were killed by decapitation and the blood was collected in a centrifuge tube. The blood was centrifuged at once and serum removed for analysis.

The livers were then quickly removed, after which the leg muscles were dissected off and freed of gross masses of fat and connective tissue. All of the tissues were weighed in covered receptacles, placed in an oven at 100°C., and dried until constant weight was attained. The tissues were then ground to a powder and returned to the oven for several days. After this, they were stored in glass stoppered flasks and aliquots taken for analysis.

Analytical methods. Serum chloride was determined by the method of Van Slyke as modified by Manery et al. (1938). The samples of dried tissue were subjected to the preliminary alkaline digestion which was suggested by Sunderman and Williams (1933). For the remaining analyses the serum and soft tissues were ashed overnight in a muffle furnace at 500°C. Platinum crucibles were used. Sodium was determined by the Butler and Tuthill modification of the Barber and Kolthoff method (1931).

For potassium the Shohl and Bennet method as modified by Fenn et al. (1938) was employed. Tissue phosphorus was determined by the procedure of Fiske and Subbarow (1925), using a magnesium oxide-magnesium nitrate fusion mixture.

TABLE 1

Analyses of rat tissues

NUMBER OF RATS	SERUM (PER LITER)				MUSCLE (PER KGM. FRESH TISSUE)					LIVER (PER KGM. FRESH TISSUE)				
	Water	Cl	Na	K	Water	Cl	Na	K	P	Water	Cl	Na	K	P
Potassium-deprived rats														
	grams	*mM*	*mM*	*mM*	*grams*	*mM*	*mM*	*mM*	*mM*	*grams*	*mM*	*mM*	*mM*	*mM*
2		82.0			774	16.1	46.7	67.1	71.5					
2	948	79.1	130	2.3	772	12.8	46.5	60.5	73.1	718	18.4	29.2	90.8	121
3	935	94.7	134	3.2	758	11.9	44.8	77.5	81.3	716	22.6	33.9	89.4	123
3	946	84.6	137	2.0	764	13.9	59.6	62.0	78.1	718	21.4	29.9	92.9	116
2	946	86.0		2.3	757	12.8	64.5	55.4	82.0	718	24.0	28.5	97.5	128
2	927	87.0	143	3.1	759	11.3	60.7	60.3	80.6	709	20.9	25.7	99.3	125
2		88.0	140	3.0	764	11.1	55.3	66.0	77.5	709	22.6	31.3	102	125
Av.	940	85.9	137	2.6	764	12.8	54.0	64.1	77.7	715	21.6	29.7	95.3	123
Control rats on purified diet														
2		102	137	6.8	764	11.5	19.5	108	79.5	706	27.6	23.4	93.5	108
2		107	141	6.9	764	11.5	17.1	110	78.5	703	29.1	24.4	96.5	119
Young rats from stock colony														
2	935	103	138	8.9	771	13.6	18.1	113.6	83.2	701	27.2	24.7	100	120
2	940	101	137	8.5	772	15.1	20.3	113.3	83.5	704	28.4	24.6	97.2	120
Adult rats from stock colony														
*		103	144	4.0	761	12.9	18.2	113		701	29.3	24.8	96.9	120
†	939	105	144	5.2	774	14.5	23.3	106	78	739	31.3	29.0	95	114

* Data reported by Fenn et al. (1936, 1939). The animals were fed calf meal. The blood values refer to plasma.

† Data reported by Harrison and Darrow (1938). It represents the average of 11 animals, which were fed Purina Dog Chow. The results are expressed per kilogram of fat-free tissue.

Because of lack of material, duplicate determinations for potassium were carried out on only half of the samples of mixed sera. All of the other figures in table 1, with but a few exceptions, represent the average of at least two analyses. The mean difference between duplicate potassium analyses was 1.5 per cent, sodium 1.8 per cent, phosphorus 1.0 per cent, chloride (on serum) 0.6 per cent, chloride (on liver and muscle) 3.4 per cent.

RESULTS. The analytical data are shown in table 1. Each horizontal row of figures represents analyses of serum, muscle and liver which refer to the same group of rats. For comparison, data obtained by other workers are also included.

It is evident that a deficiency of potassium leads to a decreased level of potassium in the serum. In the case of sodium, the average value for

TABLE 2

The amount of sodium per kilogram of tissue which is assigned to the intracellular and to the extracellular space

NUMBER OF RATS	MUSCLE			LIVER		
	Extracellular water	Extracellular sodium	Intracellular sodium	Extracellular water	Extracellular sodium	Intracellular sodium
Potassium-deprived rats						
	grams per kilo	*mM*	*mM*	*grams per kilo*	*mM*	*mM*
2	175	24.2	22.5			
2	146	19.0	27.5	210	27.4	1.8
3	112	15.2	29.6	212	28.8	5.1
3	147	20.3	39.3	226	31.2	−1.3
2	133	18.4	46.1	250	34.5	−6.0
2	115	16.7	44.0	212	30.8	−5.1
2	112	15.9	39.4	229	32.6	−1.3
Control rats on purified diet						
2	96	13.7	3.4	242	34.6	−10.2
2	100	13.9	5.6	240	33.4	−10.0
Young rats from stock colony						
2	117	16.3	1.8	234	32.8	−8.1
2	133	18.4	2.0	251	34.6	−10.0
Adult rats from stock colony						
*	111	16.2	2.0	252	36.8	−12.0
†	123	17.9	5.4	265	38.7	−9.7

* Data reported by Fenn et al. (1936, 1939).

† Data reported by Harrison and Darrow (1938).

potassium-deprived rats is not appreciably different from the figures obtained for the various groups of normal animals. There is considerable variation in the individual results. A marked reduction in serum chloride occurs as a result of the deficiency.

The livers of the potassium-deprived rats show a decrease in the level of chloride, an increase in sodium and no important changes in phosphorus, potassium or water. No adequate explanation can be offered for the low

chloride figures. A decrease might be expected in view of the blood findings. However, in the case of muscle tissue of these same animals, the content of chloride falls within normal limits. As for sodium, the differences observed between the normal and the depleted rats are no larger than the variations noted for stock animals in different laboratories (see figures from Harrison and Darrow (1938), given in table 1). Conceivably, the liver might have responded to the deficiency by suffering a decrease in size, but with no large change in its composition. However, the weight of the liver, calculated as per cent of the total body weight, is roughly the same for both deficient and normal animals.

The most striking alterations occur in the sodium and potassium content of muscle tissue. Compared to the various animals on adequate rations, the potassium-deprived rats show a decrease of almost 50 per cent in the potassium of muscle and a concomitant rise in the level of sodium which averages 290 per cent. Total phosphorus and water show no changes.

Discussion. Certain calculations on electrolyte distribution have been carried out, and the results are shown in table 2. The amount of sodium which occurs in the extracellular water per kilo of fresh tissue has been estimated for both liver and muscle. The usual assumption has been made that all of the tissue chloride found by analysis is confined to the extracellular phase, and that this phase is identical with an ultrafiltrate of serum. Manery and Hastings (1939) have described the method of calculation in detail.

It is evident that there is a very remarkable increase of intracellular sodium in the muscle tissue of potassium-depleted rats, as compared with normal animals. In the control group, almost all of the muscle sodium can be assigned to the extracellular space. This holds true also for normal rats studied by other workers (Fenn and Cobb, 1936; Harrison and Darrow, 1938; Eppright and Smith, 1938; Manery and Hastings, 1939). In the deficient animals, on the other hand, there is usually more intracellular sodium than extracellular sodium in muscle. With two exceptions, there is approximately twice as much or more than twice as much sodium within the cellular phase as there is in the extracellular fluid.

Potassium is, for all practical purposes, entirely intracellular in location in the muscles of both the depleted and the control animals. It should be noted at this point that slightly more potassium is lost by the muscles of potassium-deficient rats than corresponds to the gain in sodium. Presumably there are changes in other constituents, possibly in magnesium or calcium, which make up the deficit.

The occurrence of intracellular sodium in the muscles of rats has been reported by Eppright and Smith (1938). These workers maintained young animals on a diet generally deficient in inorganic salts for a period of 60 days. With this diet, smaller alterations were observed than were

found in the present investigation. Their average figure for muscle sodium was 19.6 mM. per kilo, of which about 6.0 mM. could be assigned to the intracellular space. According to Harrison et al. (1936), about 10 per cent of the sodium of dog muscle could not be accounted for in the same volume of extracellular water which contained the chloride. Hastings and Eichelberger (1937) have also found a small amount of "excess sodium" in the muscles of dogs, which they assigned to the intracellular phase.

A few experiments were carried out in which the muscles of the depleted animals were stimulated through the nerve. The apparatus and experimental procedure were the same as already described by Fenn (1938). It was found that the gastrocnemius muscle group of these rats was able to exert from one-half to two-thirds as much tension against an isometric lever as was the case for normal rats of the same size. However, after the first few minutes there was no evidence of fatigue during a 30 minute period of intermittent tetanus (i.e., a half-second tetanus every second). The reduced tension may have been due to impaired circulation or other conditions resulting from poor nutrition. It does not necessarily mean that the functional ability of the muscle fibers was altered by a change in their chemical composition.

The histological appearance of liver and muscle tissue in potassium deficiency has not been studied in great detail. However, in a preliminary survey no abnormalities were discovered.

No experiments were performed in which animals were serially sacrificed in order to study the time course of the changes in muscle. A few rats were killed after being on the potassium-low diet for 25 days instead of the usual 45 day period. On analysis, it was found that the potassium content of muscle fell from the normal level of 110 mM. per kilo to 86 mM. per kilo after 25 days; and after a depletion period of 45 days it was depressed to a level of 64 mM. per kilo. No further reduction could be achieved by continuing the experiment until the rats became moribund.

In the case of liver tissue, the calculation of sodium distribution leads to a kind of paradox (see table 2). Almost without exception, the actual total amount of sodium per kilo of liver is less than the calculated amount of extracellular sodium. Harrison and Darrow (1938) have called attention to this situation, which they presumed to be due to the large amount of red cells included in the samples of liver. However, Fenn (1939) has estimated the concentration of blood left in liver samples by doing red cell counts on an aqueous suspension of the tissue. When the animals were bled to death and the tissues blotted 0.7 to 3.0 per cent of blood was found to remain in the liver. This amount is quite inadequate to account for the preponderance of chloride compared to sodium which was found by analysis. It is, therefore, more logical to assume that liver contains an intracellular phase, not composed of blood cells, which contains an

excess of chloride over sodium. In the liver tissue of rabbits, according to Manery and Hastings (1939), these two elements are present in ultrafiltrate proportions. Consequently the difficulty mentioned above does not arise in this species.

SUMMARY

1. Studies have been carried out on the effect of potassium deprivation on the concentration of some of the electrolytes in serum, muscle and liver tissue of young rats.

2. A deficiency of potassium in the diet leads to a serum potassium of one-half of the normal value, and the concentration of chloride is reduced by about 15 per cent.

3. The muscles of animals deprived of potassium take up sodium in large quantities and lose potassium. In some cases the muscles become richer in sodium than in potassium.

4. In the muscles of normal rats most of the sodium is extracellular in position. However, in the muscles of potassium-deprived rats most of the sodium is confined to the intracellular phase. Potassium occurs practically entirely within the intracellular phase in both normal and depleted animals.

5. The liver tissue of the potassium-deprived rats shows no large changes in the electrolyte picture. The weight of this organ, calculated as per cent of body weight, does not become altered. There is more chloride than sodium in the liver tissue of both normal and potassium-depleted rats, although the reverse relationship holds for serum.

I wish to acknowledge gratefully many helpful suggestions from Dr. Wallace O. Fenn. I am also indebted to Miss Eugenia Sheridan for several of the analyses of potassium in serum and to Miss Lorraine Haege for a few of the phosphorus figures.

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AXON DIAMETERS IN RELATION TO THE SPIKE DIMENSIONS AND THE CONDUCTION VELOCITY IN MAMMALIAN A FIBERS

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Received for publication June 30, 1939

The problem of the relationship between the diameters of nerve fibers and the velocity at which the fibers conduct impulses cannot be considered solved until it is possible, within the range of a homogeneous group of fibers, to predict correctly the form of the action potential on the basis of the histological picture. Of all the methods as yet proposed, the reconstruction method is the most sensitive. As the method was first employed (11) it appeared to work satisfactorily on the basis of direct proportionality between conduction velocity and the diameter of the fibers. But the full range of the fiber velocities was then unknown and later attempts to apply the procedure to the complete series failed to give an acceptable result, or involved an assumption the validity of which can no longer be maintained (10).

Recent developments have been made with other methods. Blair and Erlanger observed that in frog nerves the size of single-fiber spikes varies directly as the velocity of conduction, and they argued that if the size of the spike is proportional to the cross-section of the fiber, the velocity must vary as the square of the diameter. And Zotterman came to the same conclusion after confirming their results on mammalian fibers. Reconstructions, however, in accord with the power relationship fail to match the recorded potentials (Erlanger, 1937, fig. 14).

A different conclusion was reached by Hursh (1939a), who compared the maximal velocities in a series of mammalian nerves with the respective sizes of the largest fibers. All the points relating the two properties fell about a straight line. A still different relationship was found by Pumphrey and Young for squid fibers. The diameters of the large axons in the fresh state were measured and compared with the individually determined velocities; those of the small axons were measured after fixation and compared with the maximal velocity for the group. The result was a distribution of points best connected by a curve describing the velocity as varying with the 0.6 power of the diameter.

The fact that the relationship of velocity and fiber diameter has been

set forth in the several descriptions in terms of functions as different as the square, the first power, and the square root indicates the existence of steps in the derivation of the formulations that need re-evaluation. In view of this need the present investigation was undertaken. Extensive use was made of the reconstruction method for two reasons. In the first place, the potentialities of the method as they would appear if advantage were taken of the current knowledge of the constants of mammalian fibers were unknown. And in the second place it was realized that the prevalent theories would be subjected to rigorous test. If the method failed to produce anything constructive, it could at least be expected that it would reveal which of the considerations forming the basis of the theories should be retained and which rejected.

Homogeneity of material. If the effect of the dimensions of nerve fibers upon the velocity of conduction is to be delimited, size must be the only variable, or the effect of all the variables other than size must be known. The duration of the spike has long been thought to be a factor entering into the determination of the velocity, but precise information about the nature of the relationship is lacking. Until this information is supplied, any system of fibers brought under examination for the purpose of ascertaining the effect of size upon velocity must have action potentials characterized by spikes of the same duration.

The saphenous nerve has been used for most of the observations, because the prominence of the elevations in the conducted action potential makes the nerve a favorable one for the identification of the position of the potentials belonging to given fiber groups. The elevations are best seen in the nerve of the rabbit (fig. 11). For convenience the groups are labelled[1] α, β, γ, δ. In the cat's nerve the alpha and beta elevations are fused, and the alpha and delta groups headed by velocities of about 90 and 20 m.p.s. supply the prominent features.

The specific question must be asked: Do the spikes entering into the delta elevation have the same duration as those entering into alpha? One of the first procedures that was devised for the determination of spike duration was to extrapolate the duration of an elevation back to zero distance (9). Great accuracy cannot be claimed for the method, but the procedure has in its favor the fact that it orients the experimenter forthwith concerning the relative durations of the alpha and delta spikes, and gives at one time and under the best of experimental conditions information about all the five hundred or more fibers in the delta pile. Figure 1, which shows the application of the procedure, is in a large measure self-explanatory. The action potentials as recorded at five distances of conduction are drawn with

[1] For the history of the names of the groups and of the ideas about them, the following references may be consulted: Bishop and Heinbecker, 1930; Bishop, Heinbecker and O'Leary, 1932; and Erlanger, 1937.

their base lines at ordinate positions corresponding to the distances. Projections on the base lines of the starts and ends of the alpha and delta elevations and of the start of a small predelta wave mark the times of these events. And lines drawn through the projection points give at their intersection with the line of zero distance, the time of the event in the unconducted action.

Proof of the expediency of determining the times by extrapolation is found in the fact that the lines marking the starts of the elevations pass through the origin. The first two lines, marking respectively the start and the end of the alpha elevation, subtend at the zero line a time of about 0.4 msec.,—a time which corresponds very closely with that obtained from the best single fiber records of alpha spikes. The third and fourth lines marking the starts of the predelta and delta groups again pass through the origin. The fifth line at the end of the delta elevation cuts the zero line at between 0.4 and 0.5 msec. Thus it is clearly shown that the spikes in the delta elevation must have a duration the same as, or very close to, those in the alpha elevation. Any possibility of the duration being proportional to the velocity is completely eliminated. The potential of the whole group has a duration of only 0.9 msec. at 5 cm. of conduction. Another line could have been drawn from the delta crests. It would cut the zero line at 0.15 msec., again a time which is in close correspondence to that for alpha fibers.

The opportunity for error, which the method shares with all methods involving extrapolations, is in this instance reduced by the fact that the extrapolation is effected with the aid of a straight line passing through five real points. One could not draw conclusions from small differences, but on the other hand, the indication that the alpha and delta spikes are closely similar in duration must be taken as being valid. It will be shown later that the finding is in accord with the body of evidence obtained from the measurements of single-fiber spikes.

Figure 1 contains another feature that has orienting significance. After 9 cm. of conduction, temporal dispersion has brought the delta elevation from an initial value of < 0.5 msec. to a total of 1.3 msec. As the potentials in slow fibers disperse rapidly, it follows that the elevation must represent a very narrow band of fibers. A brief calculation indicates how wide a band is to be looked for. If the spikes last 0.5 msec., the last of the impulses will be 0.8 msec. behind the first. With an initial velocity of 23 m.p.s., the final velocity, therefore, must be 19 m.p.s. If the wave starts at 4 μ and the velocity is in a linear relationship to the diameter, it will end at 3.3 μ. Thus the elevation will be produced by a band much less than 1 μ wide.

In the saphenous nerve of the rabbit the delta fibers conduct more slowly than in the cat (12.5 m.p.s. as compared with 22 m.p.s.). Extrapolations

were, therefore, made to see whether these fibers also have spikes with an alpha duration. The experiments were not as satisfactory as in the cat,

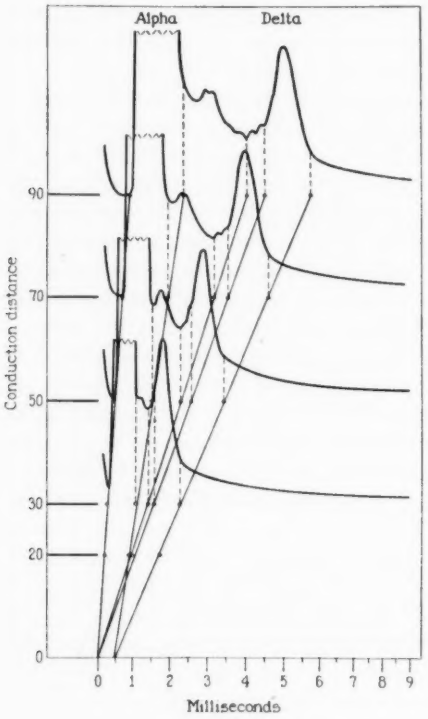

Fig. 1

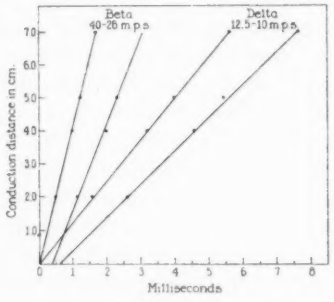

Fig. 2

Fig. 1. Action potential in the saphenous nerve of the cat recorded at the distances indicated on the ordinates. Because of crowding in the figure, only the projections of the events on the base line are shown at 2 cm. of conduction. The distance subtended by the converging lines at the axis of abscissas measures the duration of the alpha and delta spikes. The delta elevation in the tracings rises from the negative after-potential of alpha. Between alpha and delta a well defined gamma elevation is visible. It will be seen later in the reconstructions that there is always a group of fibers corresponding to it.

Fig. 2. Durations of the beta and delta elevations in the action potential of the saphenous nerve of the rabbit as recorded at four distances of conduction, and the extrapolation of these durations to zero distance. The experiment parallels the one shown in figure 1. The action potentials are omitted and only the projections of the elevations on the base lines are shown. The velocity in the fastest alpha fibers in this nerve was 70 m.p.s.

but they sufficed to show that the spikes in the two groups of fibers are similar (fig. 2). Part of the interest in the experiment lies in the fact that

the rabbit delta fibers have a velocity equivalent to the fastest B fibers in visceral nerves, but a much shorter spike duration, as the duration in the latter is about 1.2 msec. (Grundfest). The data, therefore, favor the view of Bishop and Heinbecker that there is a break in the properties of the fibers sharp enough to justify their classification into separate groups (A and B), rather than the idea that the fiber types form a continuous series. Single fiber records of rabbit delta spikes confirm the fact that these spikes have an alpha duration (fig. 3). That beta spikes also resemble alpha is shown in figure 2.

The constancy of the duration of the spikes of all fibers of the A group, regardless of the conduction velocity, also appears in series of single axon action potentials. Spikes in fibers with slow and intermediate velocities of conduction were obtained from nerves in which these velocities were at the maximum of those present. The nerves were stimulated at threshold, and the singularity of the potentials was insured by watching for indivisible constant-sized responses playing in the manner described by Blair and Erlanger (1933). Many of the nerves containing only slow fibers are short, and for that reason are unfavorable for velocity determinations, as the shock-response time is the only measurement from which the velocity can be calculated. Measurements of the shock-response time at threshold are valueless, but if the strength of stimulus is increased, a point can be found at which the time does not change with the strength of the shock. From this time it is possible to calculate a velocity that is sufficiently accurate for the present purposes.

Single delta spikes can be obtained from the saphenous nerve by using small branches and conduction distances long enough to permit clearance of the preceding groups. For these preparations, Hodgkin's method of transferring the nerve from saline into a layer of liquid paraffin was employed, after making the changes in the method necessary for its adaptation to mammalian fibers.

The recording of mammalian spikes which last but little over 0.4 msec. requires a fast amplifier, particularly if the recording is from a small strand of nerve with a high resistance. Our amplifiers have now been rebuilt by Doctor Toennies to a speed which is more than adequate for the purpose. A rectangular impulse applied through an input resistance of 100,000 ohms reaches 90 per cent of its final value in 0.015 msec. At 10 Kc the loss of amplification is only 0 to 10 per cent (as compared with 60 per cent with the former amplifiers), and at 15 Kc it is only 5 to 15 per cent, depending upon how the amplification control dials are set. An impulse with spike dimensions would have its crest delayed about 25 per cent by the old amplifiers as compared with the new,—which means that a 0.14 msec. crest time would appear to be 0.17 msec. The fidelity at high frequencies, however, brings with it one disadvantage,—the ability to record noise is also augmented, and the noise level is raised from ± 5 μv to ± 10–12 μv.

Samples of single axon spikes obtained from fibers conducting at different velocities have been collected in figure 3. They all appear alike in duration and the measurements all fall between 0.4 and 0.5 msec., without a systematic difference with respect to velocity. It would be difficult to measure them closer than to 0.1 msec.

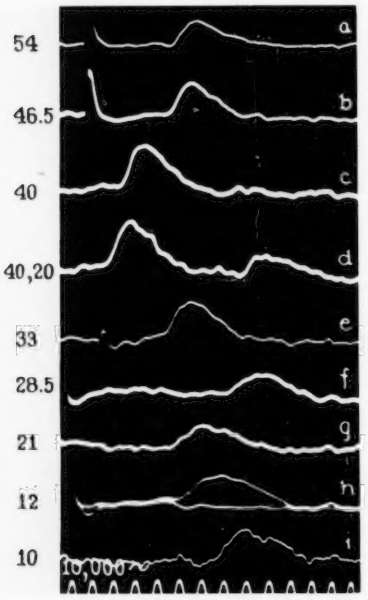

Fig. 3

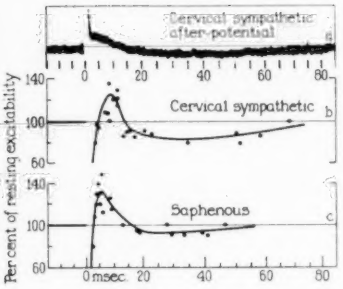

Fig. 4

Fig. 3. Single axon spikes arranged according to velocity (simultaneous responses in 2 fibers in record *i*). Record *h* shows two sweeps. Owing to the play the spike is missing in one of them. The velocities are marked at the left in meters per sec. All records have the same time scale. *a* and *b*, from rabbit cervical sympathetic; *c*, *d*, *e*, *g*, *h*, from cat hypogastric; *f*, from rabbit depressor; *i*, from rabbit saphenous.

Fig. 4. *a* and *b*, after-potential record and excitability cycle curve for the same nerve. *c*, excitability cycle curve for the delta fibers of the saphenous nerve of the cat.

After-potentials as evidence of homogeneity. The configurations of the after-potentials of the three groups of fibers, A, B, and C, are so distinctive that classification of any set of fibers under examination with one or the other group would be impossible if the after-potentials did not conform. If the delta fibers are to be included among the A fibers, their after-potentials must be the same as the alpha after-potentials, which have been taken as representative of the A group.

The after-potentials in fibers of delta velocity can be directly recorded in the cervical sympathetic nerve of the cat, as these fibers are the largest in the nerve. The potentials have an A configuration (fig. 4 a).

In the delta group of the saphenous nerve of the cat the after-potentials cannot be recorded directly, and they are even too small to have their form determined by difference in comparisons of the action potentials of the whole nerve, with and without the inclusion of the delta group in the activity. There is, however, a good indirect method for determining their form, derivative from the parallelism that exists between the after-potential and the excitability cycle. Below the action potential of the cervical sympathetic nerve shown in figure 4 a there is drawn for comparison with it (fig. 4 b) the curve of excitability as determined on the nerve by the method of size of response to a near-threshold stimulus. That the curve follows the form of the potential is readily apparent. In the saphenous nerve at long distances of conduction the separation of the delta elevation is sufficiently great to permit an examination of the excitability. A good deal of random variation occurs in the responses to near-threshold stimuli, but the variation is not so great as to prevent the form of the curve from emerging (fig. 4 c). It is like that in the cervical sympathetic nerve. Both curves have the form known to hold for alpha fibers (12) and they differ widely from the curves for B fibers (13), although the velocity in the fibers approaches a B value.

The velocities in the delta elevation of the saphenous nerve of the rabbit actually fall in the B range, and the delta fibers are so numerous in this nerve that the form of the after-potential can be directly observed. It has an A configuration.

Additional evidence that the after-potentials in all A fibers are alike is contributed by Hursh's observation that the after-potentials in immature alpha fibers, at the stage in which their impulses are conducted at delta velocity, have the form of those in adult fibers.

The medullated fibers in the saphenous nerve are within the limits of measurement homogeneous with respect to the spike and after-potentials. They differ with respect to their velocities and periods of absolute refractoriness. We have not examined the refractory period extensively, but we have had enough experience with it to know that it varies in a continuous manner, as described for frog fibers by Blair and Erlanger. The delta fibers have a refractory period of 0.6 to 0.7 msec. in the cat and 0.9 to 1.0 msec. in the rabbit, as opposed to 0.4 to 0.45 msec. for the fastest alpha fibers. In view of the constancy of the spike, the variation of the refractory period is surprising, as no previous examples have been known of exceptions to Adrian's finding that the absolutely refractory period ends at the base of the spike. The reason for the progressive divergence between the spike duration and the refractory period as the velocities de-

crease is not clear. The properties of the after-potentials cannot be called upon, as they are constant throughout. Size, however, may enter into the determination of the duration of the absolutely refractory period, just as it does into the determination of the resting threshold of excitation. The prolongation of the absolutely refractory period of immature alpha fibers found by Hursh affords another example of a modification of this property by the size of the fibers.

Preparation and measurement of fibers. The obtaining of good histological preparations depends in large measure upon having the nerve in good condition at the time of fixation. In order to make the technique as conservative as possible, the action potential was recorded as soon after isolation of the nerve from the body as enough time had elapsed to permit the preparation to come to 38°C. and into equilibrium with the gas mixture (95 per cent O_2 + 5 per cent CO_2). Immediately thereafter the nerve was fixed in 1 per cent osmic acid. The outside diameters of the fibers were measured with an ocular micrometer, and as each measurement was made the fiber was checked on a microphotograph of the section enlarged to 2000 diameters. The fibers were then catalogued according to size and the distribution was plotted to the nearest 0.1 or 0.25 μ, as seen in the charts (figs. 5, 8, 10, 12, 15).

The first question to be asked about the measured diameters is: how faithfully do they represent the diameters of fresh fibers? There can be little doubt that shrinkage occurs in the course of preparation. Hursh found that the dehydration process used during embedding shrunk the fibers 10 per cent (8.2–12.9), and Arnell had previously found a similar amount of shrinkage produced by another method. A corrective factor, however, would permit calculation of the size in the fresh state with a fair degree of accuracy, and shrinkage could only cause difficulty if it were differential. Hursh found that it was not differential for fibers larger than 10 μ. Smaller fibers were not examined.

A more serious difficulty is found in the random variation of the diameter in the course of the fibers. In 100 serial sections from 0.6 mm. of nerve Hursh found the standard deviation of a single measurement of a 6.5 μ fiber to be ± 0.47 μ. Of this amount not more than ± 0.1 to 0.2 μ could be attributed to fortuitous error in reading. Duncan found a still greater irregularity in the outline of fibers fixed in osmic acid. In parts of the distribution curves, where the number of fibers is changing slowly with the diameter, the differences between the measured diameters and the true mean diameters would tend to cancel out, as erroneously large measurements would compensate erroneously small measurements of adjacent fibers. But, as will be seen later, in positions where there is a rapid transition in the sizes of the fibers, it is possible for a deviation of the above magnitude to cause considerable difficulty in the preparation of reconstructions through making a band of fibers appear too wide.

The distribution of fibers according to size in the saphenous nerve of the cat can be seen in figures 5 and 8. The charts are similar. Both show the fibers grouped in two piles. Many indications of how the fibers will enter into the compound action potential appear straightaway on inspection of the distribution curves. Enumeration of the indications will be made, using the information contained in figure 5; but figure 8 would have served as well. It is obvious at the outset that the first pile will form the first elevation, and the second pile the second elevation. The minimum in the fibers comes at about 5 μ, and the minimum in the potential just after the first elevation; therefore, the fibers between 5 and 6 μ should form the end of the elevation. Following the minimum in the potential, the

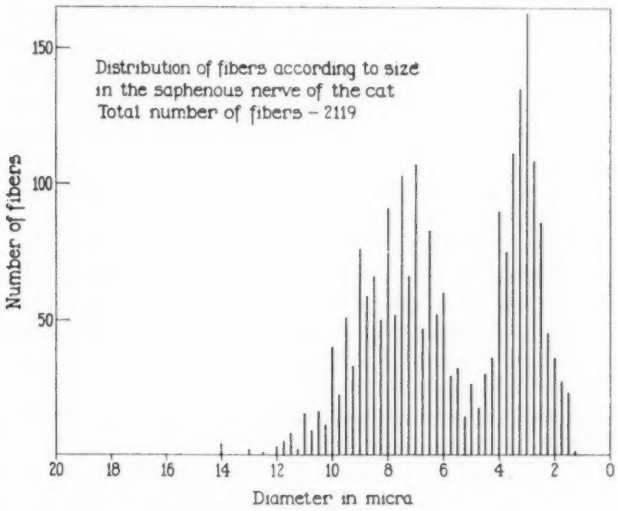

Fig. 5

potential builds up again, slowly at first and then more rapidly to form the delta peak. According to an expectation from an observation previously cited, the delta peak should be produced by a narrow band of fibers. Of all the fibers available, the group that is found in such great numbers around 3 μ appears to be the most likely one for the purpose. The elevation should start with fibers between 3 and 4 μ in diameter. As the fibers at the start of the first elevation are 14 μ in diameter, they are four times as large. Four is approximately the factor that relates the velocities of the fastest alpha fibers to those of the fastest delta fibers. Therefore, the most favorable basis for starting the reconstructions is one which considers the relationship between fiber diameter and velocity to be linear.

The method of making reconstructions is the following. After a velocity

has been assigned to a fiber of a given size, the conduction time is calculated from that velocity and the distance of conduction at which the action potential in the nerve had been recorded. A triangle imitating the dimensions of a spike and having a height proportional to the number of fibers of the size in question present in the nerve is then drawn above the base line, with its front touching the abscissa corresponding to the conduction time. After all the triangles are in place, they are added together and the resulting curve is compared with the form of the recorded action potential.

The assumptions on which the original reconstructions were made (11) were that the axon spike height is proportional to the cross-sectional area of the fiber and that the velocity is proportional to the diameter. That there is a fallacy in these assumptions soon became apparent when reconstructions were attempted on the same basis with the present data. Of the defects in the result one of the most outstanding was the fact that the second elevation was much too small as compared with the first. There could only be one explanation for the discrepancy; namely, that the assumption that the spike height varies as the cross-section of the fiber was incorrect.

At the basis of the assumption was the sound physical fact that the potential drop across a resistance, through which a current is flowing from a source that would yield the potential, E, on open circuit, depends upon the internal resistance of the source. As the resistance of the nerve fibers would vary as their cross-section, this dimension was taken to be the controlling one in the calculation of the spike heights used for the first reconstructions. At the time there was no reason for questioning the calculation, because of the apparent success with which the then known part of the compound action potential was reproduced. Nor has the calculation been questioned up to the present time.

One factor, however, was neglected. The resistance of a nerve fiber depends not only upon its cross-section, but also upon its length. Neglect of this factor is equivalent to the assumption, either that the factor is not important, or that the length is constant. The second alternative reveals the source of the difficulty. Data have just been presented showing that the spike duration is constant; and the view that the wave length varies with the velocity is, therefore, reaffirmed. If one work on the hypothesis that the velocity varies as the diameter, one must at the same time recognize that the length of the source of the potential must vary as the diameter. Thus the proper statement about the internal resistance controlling the height of the spike would be that the resistance varies directly as the diameter—because of the wave length—and inversely as the square of the diameter, on account of the effect of the cross-sectional area. The net variation, therefore, is inversely as the *first power of the diameter.*

Spike height and fiber size. Introduction of the internal resistance into

the calculation of the spike height as the first power rather than as the second makes a large difference in the values obtained. The expression connecting the spike potential, e, and the diameter, D, would have the form

$$e = \frac{R}{A/D + R} E$$

where R is the external resistance, A/D is a term representing the internal resistance, and E is the e.m.f. generated by the fiber. If the membrane resistance were included it would be in series with the internal resistance; and as it too varies inversely as the diameter, the two terms representing resistance could be combined and the form of the above expression would be unchanged. The expression is in the second degree and represents a hyperbola. However, if R is small compared with A, the graph approaches a straight line.

An idea of the relative magnitudes of R and A can be gained from the recorded potential of a single axon spike. Large axons in the saphenous nerve give potentials of about 0.1 mv. If the e.m.f. generated by the fiber is 50 mv, e/E would have a value of 0.002; and if the diameter of the axon is 10 μ, it can readily be shown by the substitution of these values in the above expression that A is about 5000 times as large as R. With a ratio of this magnitude e would come closely into linear relation with D. That the spike heights actually vary linearly with the diameters of the fibers may be concluded from the existing data on the relationship of the spike size to conduction velocity. Blair and Erlanger observed that the two properties are in linear relationship in frog fibers, and Zotterman later made a similar observation in mammalian fibers.

A few observations of our own confirm their findings. Some of them are shown in figure 6 and in figure 3 d. In the latter, two axon spikes are shown in the same record, one with a velocity of 40 m.p.s., the other with a velocity of 20 m.p.s. One spike is twice the other in size.

Blair and Erlanger, and Zotterman concluded, on the ground that the spike size would vary as the cross-section of the axon, that the velocity of conduction must vary as the square of the diameter. Another interpretation, however, is possible. If the velocity varies as the diameter, the size of the spike must also vary in this manner. With Hursh's recent data at hand it would be impossible to postulate a velocity-diameter relationship far from linear; therefore, the best working hypothesis appeared to be that the spike-size diameter relationship is linear.

When reconstructions were made with the use of linearly varying spikes, it was found that the needed area had been supplied for the potentials of the slow fibers. But the reconstructions had other faults. The delta elevation was too close to the alpha elevation. Analysis of the cause of

the proximity brought out the finding that it was attributable not only to the fact that the delta crest came a little too early, but also to the fact that the alpha crest came a little too late. It was somewhat surprising to find that temporal dispersion in accord with linear variation of the velocity with the diameter made the first elevation too broad. Not only was all possibility that the velocity might vary as the square of the diameter thereby removed, but linear variation was also called into question.

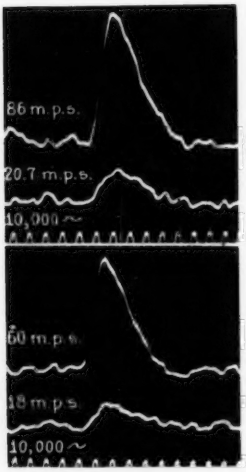

Fig. 6

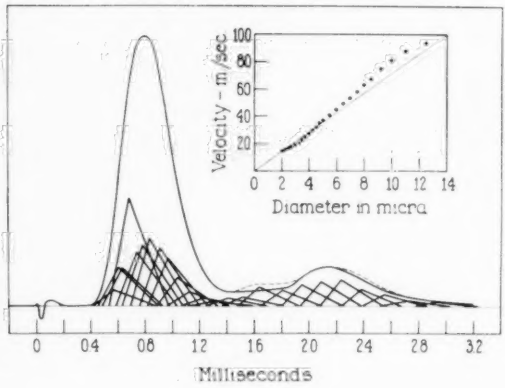

Fig. 7

Fig. 6. Comparison of the durations and heights of spikes in single axons conducting at different velocities in a branch of the saphenous nerve of the cat. The two groups are from different preparations. Upper, $Velocity_1/Velocity_2 = 4.2$; $height_1/height_2 = 4.3$. Lower, $Velocity_1/Velocity_2 = 3.3$; $height_1/height_2 = 3.8$.

Fig. 7. Reconstruction of the action potential of the saphenous nerve of the cat from the data contained in figure 5. Conduction distance 4 cm. ————, recorded potential. ----------, reconstructed potential at the places at which it does not coincide with the recorded potential. The fibers larger than 12 μ are grouped with the 12 μ fibers. *Inset*, Size-velocity curve. Each dot in the curve represents a triangle.

At this point the statement of the problem was changed. The form of the action potential was so close to emerging in the reconstructions and so good an idea had been gained about the part of the action potential to which given groups of fibers should contribute, that it was decided to locate the fibers in the reconstruction in the proper place to make the contribution, and then to calculate the velocity which would give them that position. The result would be an empirical velocity-diameter curve, which could be studied for further clues to the mechanism of velocity control.

Reconstructions. The procedure was in every case the same. The fibers were grouped according to the amount of dispersion that would take place in their potentials. For the large fibers the dispersion is small and the fibers could be taken in groups up to 1 μ wide. But for the small fibers the dispersion is very large (vid. figs. 9 and 14) and bands of 0.25 to 0.1 μ had to be used. The smallest unit was necessary, in order to prevent the appearance of a saw-tooth contour in the summation curve. The height of the triangles used in the plotting was obtained by multiplying the mean diameter of the group represented by the number of fibers. The base of the triangle was in accord with a spike duration of 0.4 msec. (0.44 msec. in figs. 7 and 16), and the crest time was set at one-third of the duration.

After the reconstruction had been completed, the velocities of conduction that would put the potentials in the positions occupied by the triangles were calculated from the conduction times. These velocities were then plotted against the fiber diameters, as is seen in the insets of the figures.

The excellence of the reconstructions could be judged by comparison with the action potential that had previously been recorded from the nerve. Certain precautions are necessary with respect to obtaining the action potential form. As is well-known, when a weak shock is used, the response appears after a latency. And when a strong shock is used, excitation spreads along the nerve away from the cathode. The ordinary saphenous nerve picture obtained with a shock strong enough to stimulate the delta fibers is distorted by the fact that the alpha elevation has been brought too far forward by the spreading shock; and the distortion is sufficiently great to be a disturbance in an analysis in which tenths of milliseconds are of significance. In order to obtain the form of the potential without distortion, the stimulus was increased with the development of the alpha elevation under observation, and a record was taken at a strength of stimulation at which the latency effect had disappeared and spread of excitation had not begun. The strength of the stimulus was then increased above the point of any latency effect in the delta fibers, and a second record was taken. The final potential form used was a composite of the two records, in which the first elevation was located from the first record and the second elevation added from the second record. The alpha elevation was adjusted in height to the height of the alpha elevation in the reconstruction and the other points were put in accordingly.

Size-velocity curves. As may be seen in a survey of the figures, all the matches between the reconstructions and the recorded potentials are good. The size-velocity curves obtained from them must, therefore, give a reliable picture of what the velocities of conduction in the several fiber groups must be. A survey of the size-velocity curves in turn brings out the fact that they have similar forms. Roughly they follow a straight line, but they

deviate from linearity in a systematic manner. The curves are all serpentine. They start with an upward concavity in the region of the small fibers and pass through a point of inflection at about 6 μ, to continue from there on with a downward concavity.

When the form of the empirical velocity-diameter curves was established, the next step was to account for their features. The first question asked was whether any part of the curve was traceable to the fact that the outside diameter instead of the axon diameter had been used in the reconstructions. Use of the outside diameter is valid only in so far as its ratio to the axon diameter is a constant. If there is variation of the ratio

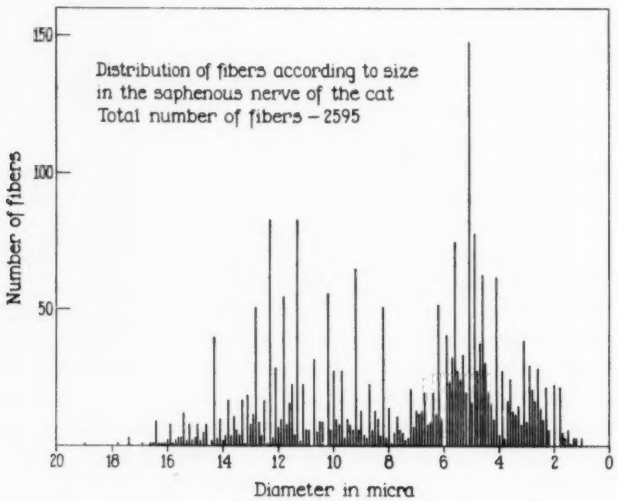

Fig. 8

with respect to fiber size, a correction for it should bring the curve one step nearer to the true form.

Ratio of axon diameter to outside diameter. A constant ratio was claimed by Donaldson and Hoke, but the more recent measurements of Schmitt and Bear and of Arnell are not in accord with their description. Arnell's data show that the myelin is relatively thicker on mammalian fibers smaller than 9 μ,—which means smaller than 8 μ in fixed preparations. As the slopes of the velocity diameter curves are greater below 8 μ, the observation appeared to be significant to our problem. If the fibers below that diameter have relatively thicker myelin sheaths, they will have relatively thinner axons and slower velocities. Therefore, when the velocities are plotted against the diameters, the line through them will not pass through the points representing the larger fibers.

A further investigation of the relationship was easily possible because suitable data were already present in our files. Doctor Ranson had given us a section of an osmic acid preparation of the saphenous nerve of the cat in which the fibers were in a nicely rounded state. In a fasciculus of this nerve containing 675 fibers, measurements of the inside and the outside diameters had been made upon a photographic enlargement; and calculation of the ratios was a simple matter. The ratios of the axon diameter to

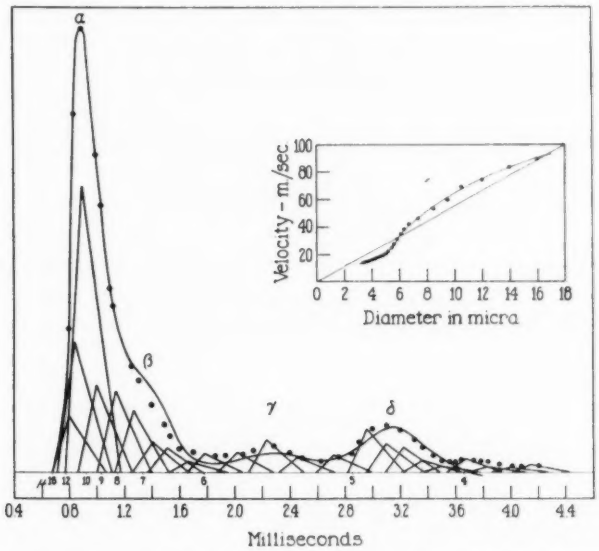

Fig. 9. Reconstruction of the action potential of the saphenous nerve of the cat from the data comprised in figure 8. Conduction distance 6 cm. ————, recorded potential. Heavy dots, summation of triangles. Note that the recorded action potential is diphasic. It undercuts the reconstruction following the first elevation, and the second elevation is so diphasic that the potential passes below the base line at the position of the 15 m.p.s. fibers. The latter, therefore, are visible in the reconstruction, but not in the potential. The fibers larger than 16 μ are grouped with the 16 μ fibers.

the total diameter are plotted in figure 12, along with the distribution curves of the fibers. Each point represents the average ratio for all the fibers of the size in the bundle. There is some scatter of the points, but it will be noted that the places at which it is greatest are those at which there are few fibers, and therefore at which there is less opportunity for the random error of individual readings to be compensated. As in Arnell's data, the myelin begins to get relatively thicker on fibers below 8 μ. Below 3 μ the scatter of the points is too great to permit drawing a curve,

and Arnell's statement that the relative thickness begins to decrease again at that point cannot be tested. Above 8 μ the ratio is practically constant at 0.69, a value corresponding with Donaldson and Hoke's figure of 0.71.

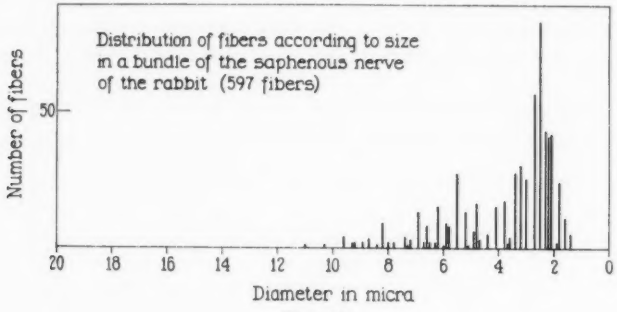

Fig. 10

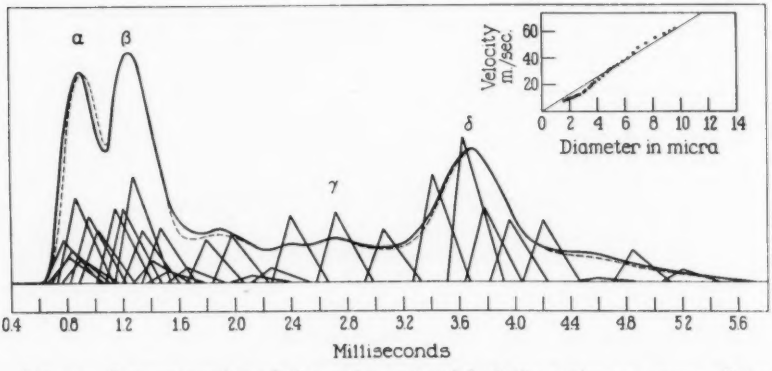

Fig. 11. Reconstruction of the action potential of the saphenous nerve of the rabbit from the data comprised in figure 10. Conduction distance 4 cm. ————, recorded potential. ----------, reconstructed potential at the places at which it does not coincide with the recorded potential. Although every column of fibers in the distribution chart recording fibers smaller than 5 μ was plotted individually, the dispersion is so great that gaps appear between the triangles. The addition is done in a way allowing an area below the summation line equivalent to the projections above it. Two fibers, one at 11 μ and one at 10.3 μ, are included with the pile at 9.6 μ.

The data contained in the ratio curve make it possible to calculate what the size-velocity curves would be like in terms of the axon diameter; and the calculation has been made for the three graphs found in the insets of figures 7, 9 and 11. The result is striking (fig. 13 A). In two of the derivative curves there is none of the initial upward concavity that made the

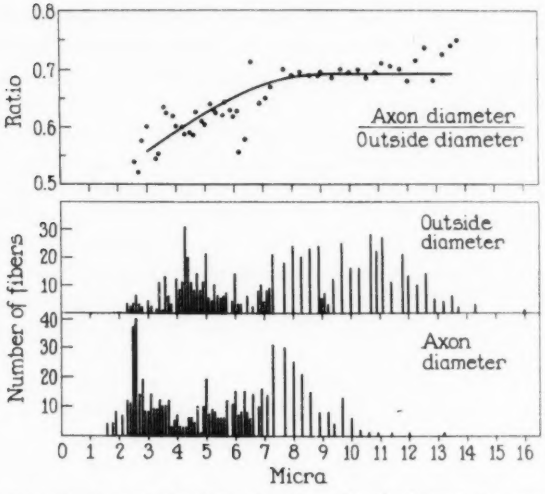

Fig. 12. Distribution of the fiber sizes in a fasciculus of the saphenous nerve containing 675 fibers, plotted for both the axon diameter and the outside diameter. In the uppermost graph the axon-diameter outside-diameter ratio is plotted against the outside diameter.

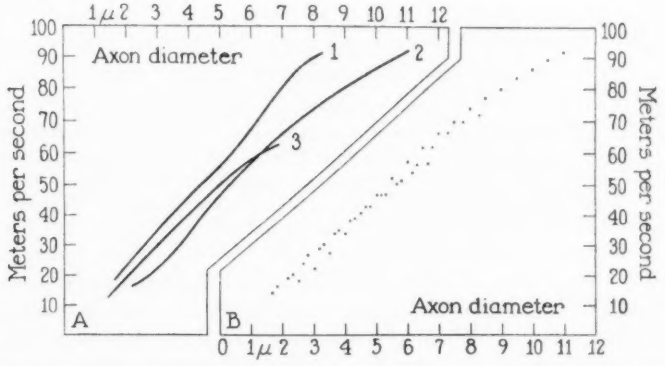

Fig. 13. A, size-velocity curves from figures 7, 9, and 11, calculated in terms of the axon diameter and presented respectively in curves 1, 2, and 3. B, curves in A brought together by multipliers. Points representing curve 2 may be identified as they fall at 3, 3.5, 4, etc. μ. Those for curve 3 occur at the same intervals, but 0.15 μ later; for curve 1 they fall 0.3 μ later.

original curves serpentine, and in the third the concavity is nearly obliterated. This residual flattening at the end of the curve may have another cause. Wherever in the distribution of the fibers there is a sharply sep-

arate band, the error of measurement will tend to make the band appear too wide; and moving the fibers back from their registered position to their proper position, in order to obtain a fit with the action potential, will flatten the size-velocity curve.

If the conclusion drawn from the adjusted curves is correct, it should be possible to construct the action potential from the axon-size distribution curve and obtain the elevations in their proper positions, when a constant multiplier is used to obtain the several axon velocities. The action potential had not been taken from the nerve analyzed in figure 12, but saphenous action potentials are so much alike that the merits of a reconstruction can be judged without having the individual potential available. The form that the potential would have after the impulses have been con-

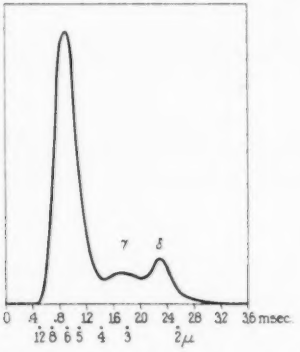

Fig. 14. Action potential which would be obtained at 4 cm. of conduction from the branch of the saphenous nerve shown in figure 12, if the conduction velocities in meters per second were 7.4 times the diameters of the axons in micra. The entries into the reconstruction in the delta region were at 0.1 μ intervals. The lower scale shows the positions of the potentials according to fiber size.

ducted 4 cm., if the velocities of the impulses in meters per second are 7.4 times the axon diameter, is shown in figure 14. The late elevations are in exactly the right place with respect to the start of the first elevation and their size is usual, except that the gamma group is a little too prominent. The principal fault is that the first elevation is too broad and has its crest too late; but this part of the potential is determined by fibers larger than 8 μ, and therefore is subject to the same difficulties that attend constructions on a linear basis when outside diameters are used.

The sum of the evidence is so greatly in favor of the conclusion that the initial upward concavity of the outside-diameter velocity curves is a consequence of using the outside diameter as representative of the axon diameter, that further investigation of the size-velocity relationship will be

confined to the derived curves. There still remains to be considered the downward concavity of that portion of the curve representing the large fibers.

Before it can be concluded that the function relating size and velocity has a curved graph, the possible objections to that interpretation must be considered. The principal objection arises from an uncertainty in the data,—the possibility that the diameter measurements may not represent the true fiber sizes. In addition to the irregularity in the outline of the fibers previously mentioned, attention must now be called to the fact reported by Arnell and evident to a lesser degree in our data, that there is variation in the thickness of the myelin on fibers of all sizes. Both of these modes of variation in the dimensions of the fibers will influence the outcome of the reconstructions in the same manner.

Suppose that there are no fibers corresponding to the stray entries ahead of the main pile of fibers in the distribution charts and that the entries are there only because of fortuitous variation in smaller fibers. If one of these entries be placed at the head of the potential and the calculation of the velocities in the other fibers be based upon it, the crest of the potential would obviously come too late. If, on the other hand, one put the crest where it should be and the proper fibers under it to produce it—as was actually done—then one would have to set back these advanced fibers from their registered positions in order to bring them within the front of the wave. The location of the fibers in the size-velocity curve would then be such that the large-fiber end of the curve would be flattened. Despite the fact that the largest fibers plotted in the graphs in figure 13 A are not the largest fibers encountered in the measurement of the nerve, but are fibers having sizes closer to those of the fibers at the start of the main pile, there is definite evidence of a terminal decrease of slope in two of the curves. The slope near the ends is different from that of the part of the adjacent curve which records fibers of the same size but which is not near the end of the curve, and it can hardly be taken to have significance as far as the size-velocity relationship is concerned.

In the same manner that fibers with fictitiously large values ahead of the crest would have to be assigned a velocity slower than their apparent size would indicate, in order to bring them under the elevation, fibers with fictitiously small values behind the crest would have to be assigned faster values. This movement again would tend to create a spurious curvature to the graph. In the saphenous nerve of the rabbit, where the first elevation is divided into two parts, the situation would be particularly aggravated. The secondary humps in the size-velocity curve of this nerve (fig. 11, inset) could also be attributed to lack of precision in the size measurements.

Whether or not there is a residual curvature in the size-velocity curve,

after allowance is made for the adventitious disturbances, cannot be stated. Against the idea that there is one is the fact that a curvature is not always

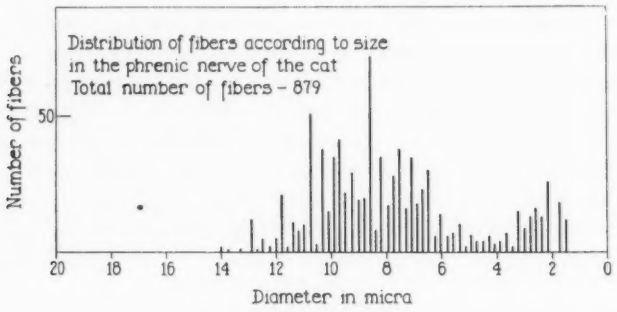

Fig. 15

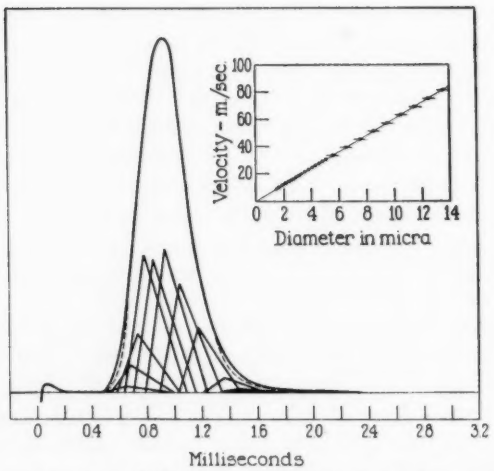

Fig. 16. Reconstruction of the action potential of the phrenic nerve of the cat from the data comprised in figure 15. Calculated for 4 cm. of conduction and on the basis that the velocity is 6 times the diameter. ————, recorded action potential. ----------, reconstructed potential at the places at which it does not coincide with the recorded potential. *Inset.* The dots give the positions of the triangles in the reconstruction, the bars through the dots the width of the band of fibers represented. xxxxxxx, fibers not plotted. Their potentials were visible at high amplification. As the strength of stimulus was raised they appeared on the crest of the negative after-potential in the region between 2 and 5 msec., which is the right place for their size.

in evidence. Figure 16 shows a perfect reconstruction of the action potential in the phrenic nerve on a strictly linear basis, and graphs 1 and 3 in

figure 13 have very little curvature that cannot be accounted for along the above lines.

The three curves in figure 13 A were brought together by multipliers, in order to visualize, as well as can be done with present data, the form of the axon-diameter velocity curve when freed from the idiosyncrasies of the individual curves. The points are plotted in figure 13 B. The result is as close to a straight line as are Hursh's data with another method. It describes the velocity as being 8.7 times the axon diameter. This is the factor used for the reconstruction of the phrenic nerve in figure 16. The significant features of the curve are all produced by fibers larger than 8 μ, and consequently the ratio 0.69 would apply. The factor 6 for the outside diameter is converted by it to 8.7 for the axon diameter.

A close examination of figure 13 A, however, reveals that the points fall along a line having a slight curvature. The line is not straightened by plotting it on logarithmic coördinates, and therefore it is not exponential. Its form is unknown. All that can be concluded at the present time is that in so far as the velocity is not a linear function of the diameter, it does not vary from linearity in the direction of being a power greater than one. It has been seen that empirically the relationship of the spike height to axon size is linear, although theoretically it is a function with a curved graph. And the velocity relationship may similarly be represented by a curve approaching a straight line.

The region in which the myelin sheath begins to increase in thickness relative to the axon is the one in which its composition according to the birefringence data of Schmitt and Bear begins to change. The spike duration and the after-potential configuration remain constant and the velocity follows the axon diameter. Therefore the properties that may be related to the characteristics of the sheath as such remain to be determined.

SUMMARY

From charts showing the distribution of fibers according to size in the saphenous nerve of the cat and the rabbit, reconstructions of the form of the conducted action potentials were made. Good fits were obtained with the recorded action potentials from the same nerves, and the medullated fibers of all sizes were accounted for. From these reconstructions empirical size-velocity curves were prepared.

The following evidence was obtained that the medullated fibers of these nerves constitute a homogeneous system and therefore are suitable for study of the variations dependent upon size.

a. The spikes all have the same duration within the limits, 0.4 to 0.5 msec.

b. The fibers all have the same after-potential system.

Success in the reconstructions depended upon the determination of the

fact that the spike size varies as the axon radius, and not as the square of the radius, as previously held.

Measurements were made of the axon diameters and the outside diameters of the 675 fibers in a fasciculus of the saphenous nerve, and the ratios of the two diameters were plotted against the outside diameters. For fibers larger than 8 μ the mean ratio was found to be approximately constant. Below 8 μ it decreased progressively. With the aid of these data axon-diameter velocity curves were calculated from the outside-diameter velocity curves.

The velocity of conduction was found to be approximately proportional to the axon diameter, but a slight curvature in the graph connecting the two variables indicated that the velocity may change somewhat less rapidly than it would if the relationship were strictly linear.

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